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Editor

ROY WALDO MINER

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BEDROCK GEOLOGY OF RHODE ISLAND*

By ALONZO W. QUINN†

Introduction

The information and conclusions here presented concerning the bedrock geology of Rhode Island are based on information extracted from earlier studies (Quinn and Swann, 1950) and on results of a current systematic survey of the bedrock formations, the surficial features, and the ground-water resources of Rhode Island. The present survey is a cooperative project between the United States Geological Survey and the Rhode Island Development Council. Thanks are due W. B. Allen, of the United States Geological Survey, Ground Water Branch, for the use of FIGURE 1.

Topographic Relations

A dominating feature of the bedrock of Rhode Island is the Narragansett Basin of conglomerate, sandstone, shale, and meta-anthracite. This basin underlies the eastern one-third of the State, except for small areas near the southeast corner. The other two-thirds of the State, including the western part and the southeast corner, is underlain by plutonic and metamorphic rocks. The sedimentary rocks of the Narragansett Basin are relatively weak in resistance to erosion, so the Narragansett structural basin is also a topographic basin. Narragansett Bay is the lowest drowned portion of this lowland. The contrast between the sedimentary rocks of the Basin and the crystalline rocks of the uplands has marked topographic expression in escarpments along both borders of the Narragansett Basin.

Age Relations

The sedimentary rocks of the Narragansett Basin contain many plant fossils that indicate a Pennsylvanian age (Knox, 1944; Lesquereux, 1889; Packard, 1889, 1900; Round, 1924, 1927; Shaler, Woodworth, and Foerste, 1899). Other rocks of the area cannot be assigned definite ages, but they can be divided into five main groups on the basis of relative age. Of these main groups, only the Westerly granite is younger than the Pennsylvanian sedimentary rocks. The older groups include, from oldest to youngest, (a) several gneissic formations, (b) the Blackstone series of metamorphosed sedimentary rocks and greenstone, (c) the Esmond granite and related intrusive rocks, and (d) the Quincy granite and related volcanic rocks. In addition, there are several thin mafic dikes, of which a few seem to be younger than the Pennsylvanian rocks and others appear to be older.

The relative ages of the groups are indicated by the structural relationships of each of the groups to the Pennsylvanian strata and to each other. More specific, but still tentative, assignments of the groups to periods and eras are suggested by regional correlations.

The Westerly granite and associated pegmatites intrude folded Pennsylvanian sedimentary rocks along the southwestern shores of Narragansett Bay (Loughlin, 1910). Evidence in Connecticut seems to indicate that the Westerly granite is older than the Triassic rocks of the Connecticut Valley. A late Pennsylvanian or a Permian age is suggested.

Granite porphyry closely related to the Quincy granite is known to be older than the Pennsylvanian rocks of the Blue Hills of nearby Massachusetts because conglomerate there of Pennsylvanian age contains boulders of the porphyry (Quinn and others, 1948, 1949). A Mississippian age is probable (Emerson, 1917).

The Esmond granite and affiliated plutonic rocks are considerably older than the Quincy granite and associated volcanic rocks, as is shown by Esmond granite boulders in the volcanic rocks near East Greenwich (Quinn, 1952) and by intrusive relations of the Quincy granite to the Esmond granite in the Pawtucket quadrangle. Regional considerations suggest a Devonian or earlier age (Emerson, 1917).

Rocks of the Blackstone series were folded and metamorphosed before the Esmond granite and related rocks were intruded. That they may be pre-Cambrian is suggested by the fact that the Blackstone series is more metamorphosed than the fossiliferous Cambrian rocks at nearby Hoppin Hill in North Attleborough, Massachusetts. Still

* This paper was presented at a meeting of the Section on May 4, 1953; its publication is authorized by the Director, U. S. Geological Survey.

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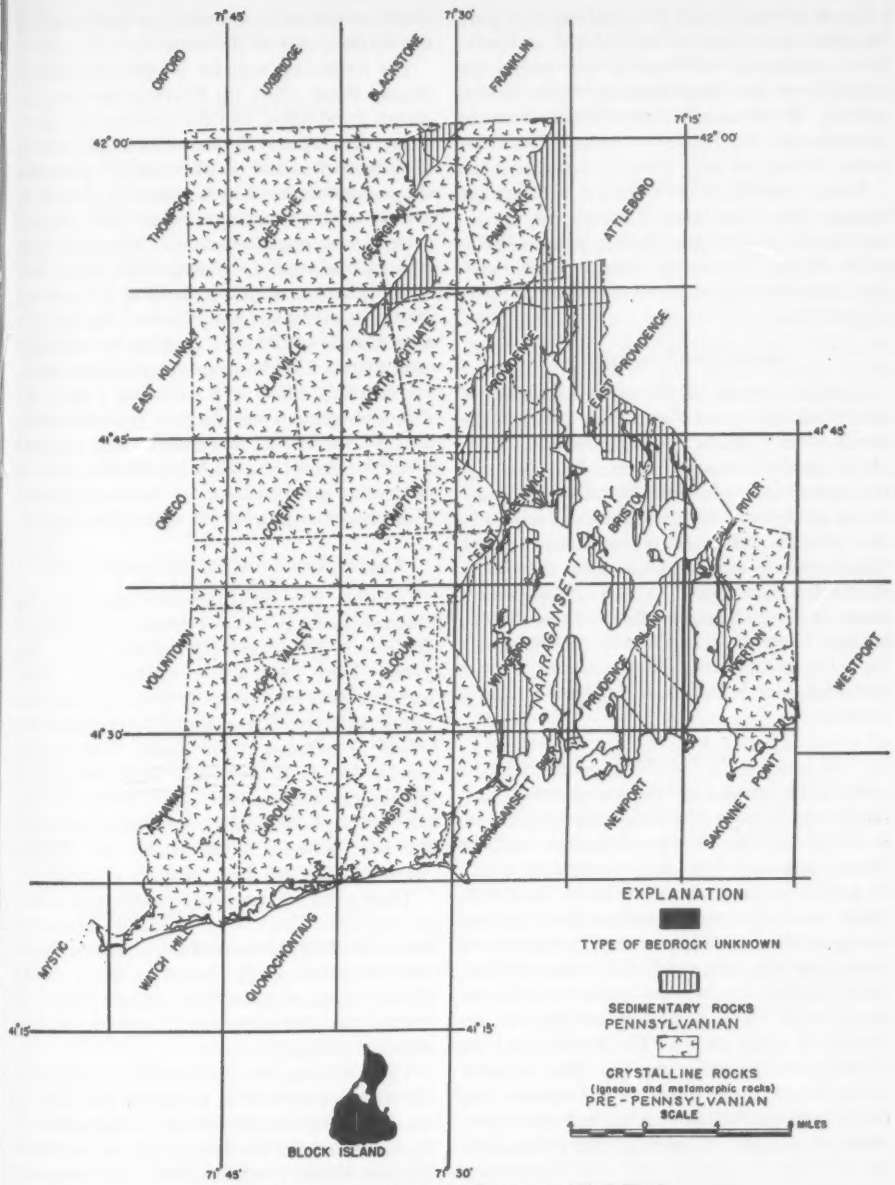

FIGURE 1. Index map and generalized geologic map of Rhode Island.

further evidence of the age of the Blackstone series is gained from the cumberlandite body near the northeastern corner of the State. The cumberlandite appears to be intrusive into the Blackstone series and into gabbro related to the Esmond granite. The helium method gave an age determination of 1,500,000,000 years for the cumberlandite (Hurley and Goodman, 1943). This

places it far back in the pre-Cambrian and gives the Blackstone series an even greater antiquity. Many geologists, however, do not accept the reliability of age determinations by the helium method. From these several evidences it seems probable that the Blackstone series is pre-Cambrian, although an early Paleozoic age is possible.

Recent mapping in the Georgiaville quadrangle suggests that three gneissic formations lie unconformably beneath the Blackstone series (Richmond, 1952). The gneissic formations are, therefore, considered to be pre-Cambrian or of early Paleozoic age.

General Structural Relations

A striking feature of the geologic structure of Rhode Island is the discordance between the trends of the Narragansett Basin rocks and those of the pre-Pennsylvanian rocks. The trends of the older gneisses and of the Blackstone series are to the northwest. Both the intrusions related to the Esmond granite and those related to the Quincy granite are discordant with these older structures. The trends of the Pennsylvanian strata of the Narragansett Basin are northward, strongly discordant with the older folds and with the intrusive contacts. These relations indicate that there have been at least two deformations in this area.

Older Gneisses

The older gneisses of the Georgiaville quadrangle include, from oldest to youngest, the Nipsachuck gneiss, the Absalona formation, and the Woonasquatucket formation (Richmond, 1952). In part, they may be correlated with the Northbridge granite gneiss of Massachusetts. They are composed chiefly of feldspathic gneiss, but also of schist, quartzite, and amphibolite. Microperthite, albite, quartz, biotite, and garnet are present almost everywhere; amphibole and kyanite are present in a few places. The bedding and the foliation are generally parallel. The Absalona formation, consisting chiefly of a distinctive porphyroblastic gneiss, has been mapped over several miles of a rather complicated outcrop pattern.

Blackstone Series

The Blackstone series includes metamorphosed sedimentary rocks and greenstones, all well exposed in the valley of the Blackstone River of the Pawtucket quadrangle (Quinn and others, 1948, 1949), as well as in several other quadrangles in the northern part of the State.

The formations included in the series are the Mussey Brook schist, the Westboro quartzite, the Sneech Pond schist, and the Hunting Hill greenstone. The Mussey Brook schist is the oldest of these formations and the Hunting Hill greenstone the youngest, except that the extrusive parts of the greenstone are contemporaneous with different parts of the other formations. The main types of rock included are fine-grained quartz-mica schist, quartzite, several varieties of intrusive and extrusive greenstone, and marble. The series is approximately 10,000 feet thick in the Pawtucket quadrangle. The bedding and parallel foliation are generally tilted at a moderate angle. The degree of metamorphism is low- to middle-grade, as is indicated by the fine texture of the rocks and by the presence of amphibole and of minor amounts of chlorite, garnet, biotite, muscovite, and albite. The marble is quarried for agricultural use.

Esmond Granite and Related Intrusive Rocks

Intrusive into the Blackstone series are the Esmond granite and related gabbro, diorite, quartz diorite, and granodiorite (Quinn and others, 1948, 1949). These rocks are confined to the northeastern part of the State. It seems probable that the Scituate granite gneiss and the cumberlandite should be included in this group. The Esmond granite has been correlated with the Milford granite (Emerson and Perry, 1907; Emerson, 1917), but this correlation cannot be substantiated until the area between Rhode Island and Milford, Massachusetts, has been mapped in detail.

These rocks are commonly massive with schistose borders. They cut discordantly across the beds of the Blackstone series and contain abundant small inclusions of the Blackstone series. Many of the inclusions have been rotated within the granitic rock, indicating that the granitic rock was implaced as liquid.

These early plutonic rocks are characterized by the large size of many of the intrusive bodies, the presence of biotite, the absence of amphibole and pyroxene, and the fact that most of the plagioclase has been altered to albite, epidote, and muscovite. In contrast, the younger Quincy granite and granite porphyry are distinguished by the small size of the intrusions, the presence of riebeckite and aegirite, and the lack of alteration of the plagioclase.

Formerly the Esmond granite was quarried extensively northwest of Providence, but it is not now being worked.

Scituate Granite Gneiss

A current problem is the determination of the relation and age of the Scituate granite gneiss of western and southwestern Rhode Island. It was formerly included with other gneisses, granites, and migmatites in the Sterling granite gneiss of late Carboniferous or post-Carboniferous age (Emerson, 1917). The Sterling granite gneiss is named for Sterling, Connecticut (Rice and Gregory, 1906). The granite gneiss of the North Scituate and Georgiaville quadrangles (Scituate granite gneiss of present usage) appears to be older than the Pennsylvanian rocks of the Narragansett Basin and therefore should not be included with the Sterling granite gneiss (Quinn, 1951). Mapping during the next few years is expected to reveal the age relations of these gneisses, granites, and migmatites.

Cumberlandite

Near the northeast corner of the State is a small hill of the peculiar rock cumberlandite (Johnson, 1908). It appears to be a small body intrusive into the Blackstone series and into gabbro related to the Esmond granite. Cumberlandite is composed of olivine, labradorite, and an intimate intergrowth of magnetite and ilmenite, along with a great deal of serpentine and other alteration products. This rock was mined for iron during colonial days, but it contains too much titanium to be mined economically for iron today.

Quincy Granite and Related Rocks

The granite and granite porphyry that compose several of the prominent hills in the northern part of the Pawtucket quadrangle are correlated with rocks in the Quincy, Massachusetts, area because of close similarity of mineral composition, textures, and structural relations (Quinn and others, 1948, 1949). The presence in both areas of the uncommon minerals riebeckite and aegirite is especially notable. The Quincy granite was formerly quarried extensively in the Pawtucket and Franklin quadrangles.

The East Greenwich group of the East Greenwich quadrangle (Quinn, 1952) has been correlated with the Quincy granite and related volcanic rocks. The East Greenwich group includes, from oldest to youngest, Spencer Hill volcanics, metadiorite, Maskerchugg granite, granite porphyry, and Cowesett granite. They appear to be younger than the Esmond granite and to lie unconformably beneath the Pennsylvanian rocks of the Narragansett Basin.

Pennsylvanian Series

The Pennsylvanian rocks of the Narragansett Basin consist of conglomerate, sandstone, shale, and meta-anthracite, all of which appear to have been formed in a terrestrial basin of deposition, as is indicated by the variable texture—fine clay to coarse boulders—the abundant cross-bedding, and the many fossils of land plants (Shaler, Woodworth, and Foerste, 1899). Similar conglomerate and sandstone in the Georgiaville and North Scituate quadrangles have been correlated with the Bellingham conglomerate of Massachusetts and with the sedimentary rocks of the Narragansett Basin on the basis of similar lithologic characters and similar structural discordance with the older crystalline rocks. These areas of conglomerate and sandstone form two smaller structural basins approximately 6 miles west of the Narragansett Basin (Quinn, 1951; Richmond, 1952). The north area is known as the Woonsocket Basin.

The Pennsylvanian rocks of the Narragansett Basin have been divided into several formations. The Pondville conglomerate is at the base of the Pennsylvanian at several places along the west and northwest part of the basin. In the East Greenwhich quadrangle it is approximately 100 to 160 feet thick (Quinn, 1952). The red Wamsutta formation, composed of conglomerate, sandstone, and shale, is exposed in the northern part of the basin, chiefly in Massachusetts. Sheets of basalt, rhyolite, and felsite are closely associated with it. The Wamsutta formation may be in part equivalent to the Pondville conglomerate, but it is chiefly equivalent to the Rhode Island formation, which overlies the Pondville. The Rhode Island formation is a very thick series of gray to black conglomerate, sandstone, shale, and meta-anthracite that forms most of the southern part of the Narragansett Basin. Some of the coarse conglomerate in the southeastern part of the basin has been correlated with the Dighton conglomerate, which is considered the youngest formation of the Narragansett Basin (Emerson, 1917). The Dighton conglomerate is widely exposed in Dighton and other parts of nearby Massachusetts.

The meta-anthracite has long attracted attention as a possible fuel and, according to Ashley (1915), was successfully used for smelting copper from 1860 to 1880. One mine is producing foundry graphite in 1953. The chief difficulties in the use of the meta-anthracite for fuel are the high ash content and the high degree of metamorphism.

The metamorphic grade of the Pennsylvanian rocks increases to the south. In the northern part of the basin the rocks are essentially unmetamorphosed. Just south of Providence the shale beds have a slaty cleavage and contain chloritoid. In the southern part of the basin slaty cleavage is prominent and garnet and staurolite are abundant. Some of the beds near the mouth of Narragansett Bay have been metamorphosed to muscovite schist. In the conglomerate, especially in the southern part of the basin, the pebbles have been elongated by metamorphism.

The Narragansett Basin has a synclinal structure, trending north through Rhode Island and northeastward into Massachusetts, but the details of structure are not well known, owing to the absence of key beds suitable for mapping and to the widespread covering of glacial deposits. In areas of continuous exposure, folds and faults are numerous enough to suggest that the structure of the basin is complex. The deformation of the Narragansett Basin was part of the Appalachian Revolution.

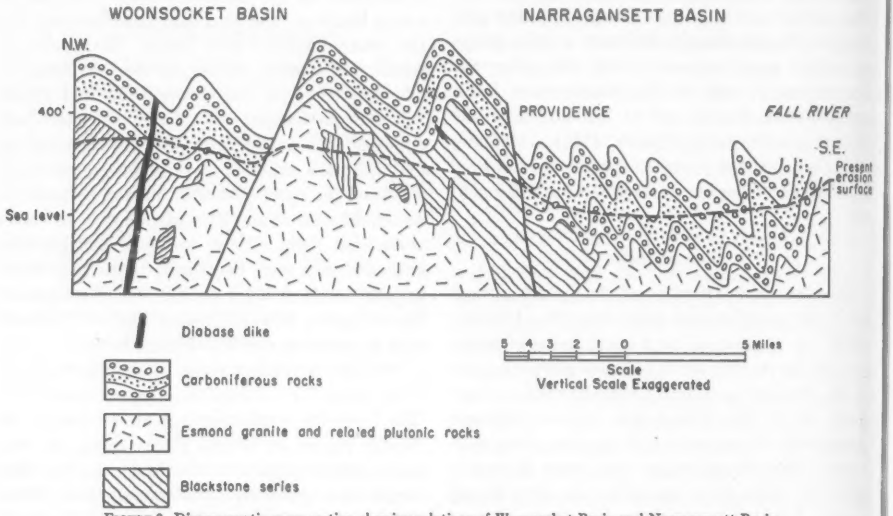

FIGURE 2. Diagrammatic cross-section showing relations of Woonsocket Basin and Narragansett Basin.

Westerly Granite

The Westerly granite is found chiefly in the Ashaway, Carolina, and Watch Hill quadrangles, near the southwest corner of the State, and in the Narragansett Pier quadrangle, at the mouth of Narragansett Bay. It includes many small pegmatites and medium-grained and fine-grained rocks ranging in composition from that of a granite to that of a granodiorite. In the Narragansett Pier quadrangle the medium-grained rocks and associated pegmatites were intruded into folded Pennsylvanian strata of the Narragansett Basin (Loughlin, 1910). Evidence in Connecticut seems to indicate that the Westerly granite is older than the Triassic rocks of the Connecticut Valley.

Both the medium-grained and the fine-grained rocks are in rather small bodies and were probably formed in the same episode of intrusion. The fine-grained granitic rock forms dikes intrusive into the medium-grained rock. These dikes are extensively quarried for fine-grained monumental stone.

Mafic Dikes

A very few mafic dikes are found in Rhode Island. Some are distinguished by an unaltered character and by being intrusive into the Penn-

sylvanian rocks or into the Westerly granite. Others are much altered and are known to be intrusive only into pre-Pennsylvanian rocks. It seems probable, therefore, that the dikes are of at least two different ages. Some of the younger dikes are diabase and are tentatively correlated with the diabase of Triassic age of the Connecticut Valley.

Block Island

Block Island is composed of a great variety of unconsolidated sediments of Pleistocene and Late Cretaceous age, and no indurated bedrock is known (Woodworth and others, 1934).

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SECTION OF PHYSICS AND CHEMISTRY

RECENT ADVANCES IN INTERFEROMETRY*

By RAYMOND JONNARD†

The refraction of light, or paradoxical breaking of its linear trajectory at the surface of transparent media, has attracted the attention of natural philosophers since early antiquity. Reflection phenomena, linked with rectilinear propagation, were described in Plato's "Timee," Euclid's "Optica" (Vol. VII), *circa* 325 B.C., in Apollodorus's "Philosophy," and in Claudius Ptolemy's "Physics" (2nd. Century A.D.). Simultaneously, the apparently contradictory notion of refraction was neatly distinguished by Posidonius, and by Cleomede‡ (*circa* 80 B.C.). It was well treated in Lucius Annaeus Seneca's "Natural Questions" (*circa* 25 A.D.), but the phenomena involved were already sufficiently popular for Aristophanes to utilize them in his theater plays (124 B.C.).**

* This paper, illustrated with lantern slides, was presented at a meeting of the Section on May 5, 1953.

† Assistant Director of Laboratories, Paterson General Hospital, Paterson, N. J.

‡ ". . . the visual (sun) ray in its direction is not flexed (when high above the horizon), while the ray directed toward the horizon is of necessity flexed, traveling through a denser air."

** The character Strepsiade proposes, indeed, to melt the waxed tablet upon which the court clerk wrote his sentence, by placing himself in the sun with a "ualos" in his hand. "Ualos" designates the druggists' transparent pebbles used to light the fire from the sun rays. (Clouds; Act II, sc. 1).

These achievements took full significance after Aristotle postulated that light is a vibrational phenomenon traveling with a finite velocity through fluids of definite densities. Thus, marked progress was made without the benefit of a solution to the secular dispute between Pythagorean proponents of light as a fluid emitted by the eyes to capture their object, and Apollodorus's disciples who admitted the existence of light *per se*.

The earliest, and most explicit, mathematical theory of refraction was given in Ibn-Al-Haitham's "Treatise of Optics" (7th. Century A.D.), after this author recognized the limitations of Ptolemy's elementary law, and his discovery of the inverse return of spherical waves, a concept which is the basis for the design of modern prism refractometers. Thus, contrary to common assertions, the discovery of refraction is considerably anterior to Tycho Brahe, Vitellio and Kepler, although this last author must be credited with the construction of the first crude refractometer[43] with which he discovered the internal reflection in glass. Laboring on the retrograde assumption of an infinite velocity for light, Descartes in 1644 produced the "sin. law," which summarizes so well most of geometric optics, sin. i/sin. $r = n$, where n is the refractive index. But we are indebted to Pierre de Fermat for correcting the fundamental error of Descartes, and for the mathematical relation which made modern optics possible, sin. i/sin. $r = V_1/V_2$. This he did by showing that the "sine law" could be deducted from the philosophical principle of "optical extremum path," or more exactly of "stationary time" *viz*: $\delta \int nds = 0$ where nds is the optical path, and n is the cartesian refractive index.* Today, it is admitted that if n varies little and *continuously*, all the rays of wave propagation of geometric optics form a totality of curves orthogonal to a family of wave surfaces. These rays are no more than trajectories of energy, and de Broglie remarks[3] that, in this case, the treatment remains compatible with *both* Newton's strangely modern corpuscular theory and Fresnel's undulatory theory. But if n varies suddenly, diffraction takes place and the concept of "ray" becomes meaningless. Thus, the determination of refractive indices may be accomplished essentially in two ways: (1) by application of the Descartes formula, involving angular measurements. This is done with the various types of prismatic refractometers and derived methods; (2) by application of Fermat's formula. This concerns *all* the interferometric differential methods.

From this point on, it is a matter of text book record that the development of instruments based on the later formula closely followed the fluctuating fortunes of the various theories of light.

Nothing remains of the pioneer work of Robert Hooke,* or that of Grimaldi (*circa* 1665) and his production of interference fringes in the *camera obscura*.† But some of the original equipment of Newton, Th. Young, Soleil (*circa* 1810), Augustin Fresnel, Mascart, Fizeau, the *two* interferometers of Jamin,[20, 21] that of Desains, of Billet, the two instruments of Sagnac[47, 48] and more recently those of Lord Rayleigh,[46] and of Le Chatelier, are jealously preserved in foreign collections. It was on the basis of the results of Young's experiment, repeating that of Grimaldi, that Fresnel, making it an *experimentum crucis*, was forced to transform his first scalar theory of light as a longitudinal elastic wave into his second, vectorial theory of transverse vibrations, capable of encompassing the then newly discovered polarization of light.

General Principles of Design

In all interferometers, two "synchronous" sources of light separated by a distance a, send two rays of wave length λ on the same point c on a plane at a distance D from the sources, each ray passing respectively in one or the other media being compared. On the plane containing the point c is produced an array of lines, alternately bright and dark, the "fringes" separated from one another by a distance h or interfringe given by $h = D\lambda/a$. Interferometers differ by; (a) the means of producing the two sources of light, (b) the means of separating the rays, (c) the means of observing the fringes or of measuring their width. The selection depends largely on the practical application in view. From a practical standpoint,

* The theosophic connotations of Fermat's principle are usually presented as a laughable matter, contrary to the teaching of contemporary physics. In reality, it was for reasons of "philosophical economy" that the principle was formulated. In turn, it proved much broader than the experimental data available at the time, and its influence in science may be felt in the work of Leibniz, Hamilton, Gauss, and even Mach. This lends support to the remark of Destouches[15] that "the essential, in order to build a theory, is to possess simple and schematic ideas of origin, rather intuitive than experimental, and a long work of the mind is needed".

* We are indebted to the Assistant Secretary of the Royal Society, the Secretary General of the Faculties, Cambridge University, and the Superintendent of the Science Museum, South Kensington, for an exhaustive search of the collections of these Institutions as well as of the Cavendish Laboratory, the Whipple Collection, and the Lewis Evans Collection at Oxford. Gunther's book, "Early Science in Cambridge, does not mention this instrument at all.

† It was Grimaldi who made first the statement "light added to light produces darkness."

it is convenient to distinguish the instruments in which the rays are more or less superimposed, and those in which they are neatly separated. Instruments of the first type are convenient for metrological applications. Those of the second type are preferable for studies on transparent media and it is with them that this paper is especially concerned.

Interferometers with widely separated beams are derived essentially from three basic designs; (1) the Young's twin apertures from which the so-called Rayleigh interferometer was derived, (2) the Jamin design in which the separation is obtained by reflection on the two faces of a thick glass plate, and (3) the Michelson design. More recent designs, however, notably the Lotmar[44] interferometer, combine certain features of both the Jamin and the Michelson types. Instruments based on Young's experiment are the simplest. The William's modification of the Rayleigh interferometer is popular in Europe. The Haber-Lowe instrument (Zeiss, Mfg.), and popularized by Hirsh,[18] is well known. The instrument that we designed in 1934 (Jobin-Yvon, Mfg.) embodies new features resulting in a compensation of the defects of certain parts, by defects of opposite sign in other parts, resulting in a greatly simplified construction, much reduced cost, greater ease of manipulation and increased accuracy.[22, 23] This instrument included only common optical microscope parts and did not require any specially corrected glass surfaces for satisfactory performance.

The Orthoptic Interferometer

All instruments listed above suffer from great bulk, relatively high initial cost, and they require some patience for their adjustment. Further marked improvements were derived from a consideration of the theory of the marginal rays normally produced in the compound microscope.[23] These rays satisfy well enough to the condition of coherence required for the production of interference fringes. The experiment entirely substantiated the derived assumption that it should be possible to make a microscope perform some of the functions of an interferometer. The result of these studies is an extremely simple instrument of small size and weight (6″ x 5″ x 5″ and 1¼ lb.), giving two well separated rays, shown in FIGURE 1. It fits on the stage of any commercial microscope thus transforming the latter into an extremely sensitive and at the same time portable and rugged interferometer.* The new instrument still embodies several features of our former type (1934), notably the differential cell block. It also includes the Jamin compensator with its tangent screw, except that now both plates are secured to the shaft, resulting in an extended linear scale. One of the main advantages of the new design lies in the independence of the position of the interference pattern from that of the light source, so that nearly any external source, including a sunny sky, may be used.

Considerations relative to the practical construction, the numerical aperture, the illumination requirements, the choice of the microscope to be used conjointly, the optimum characteristics of the optical parts required, and the elementary theory were previously discussed. The following complementary comments are now in order.

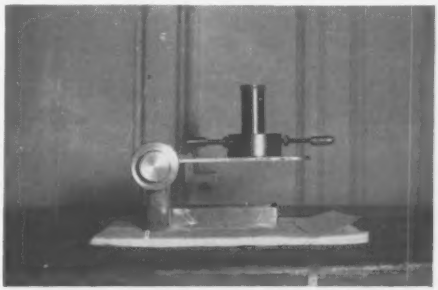

FIGURE 1. The microscope interferometer, unmounted.

The theory indicates that the entrance aperture should have a width of about 0.046 mm. in order to produce a satisfactory field of view. Similar conclusions are reached in a different way. According to Fresnel's theory, the "effective width" q of the first diffraction fringe produced by a single slit b mm. wide placed in front of a lens whose focal distance is f, is given by $q = 0.886\lambda f/b$. This relation gives the dimensions of each of the twin slits required to produce a diffraction fringe of a size comparable to the diameter of the image of the entrance aperture of the instrument, this is found to be about 0.05 mm. for best results, but may be considerably larger. Subsequently, if one considers the net effect of two such slits separated by a distance a mm., one finds that the spacing of the fringes which appear on the diffracted image is, $h = kf\lambda/a + b$. These relations are useful in

* Manufactured by American Instrument Company, Silver Spring, Md.

determining the optimum dimensions of the mechanical parts of the instrument, and in avoiding those critical conditions which result in the disappearance of some or all of the fringes (when b/a is an integer), or which produce extraordinarily hazy fringes (when $b = kf\lambda/a$). The latter effect must not be confused with the "Sprung" or "jump" of a fringe in filtered light, although *both* may be produced or even co-exist when certain combinations of dispersive media are placed in the cells. Furthermore, one demonstrates (Dyson[16]) that an axial shift of the wave front results when two media of equal thickness e, and different refractive indices n_1 and n_2 are compared. This shift is given by $t = e(n_1 - n_2)/n_1$. One demonstrates that the fringes have the maximum sharpness (coherence close to 1, when defined by Zernicke's formula) when the following relation exists $t = \lambda n_1/A^2 n_2(n_1 - n_2)$, or $e(n_1 - n_2)/n_1 = \lambda/n_2 A^2$, where A is ½ the angle of aperture of the (decolimating) lens, and the quantity $\pm\lambda/2n_2A^2$ is its depth of focus. Thus in some cases it may be preferable to forgo the advantages of the wide range of measurements possible with the instrument and to limit the comparisons to a rather small value of dn, in order to keep the coherence close to its maximum value, since the conditions of narrow slits and great focal depth are mutually irreconcilable.

A functional analysis of the instrument[22, 23] reveals that the following factors contribute to make the final error in the measurement of refractive index differences: (1) fluid layer thickness; (2) cell faces parallelism; (3) wave length uncertainty, and light dispersion; (4) apparent color temperature of the source; (5) mode of pointing of the fringes; (6) value of the compensator calibration; (7) error on the compensator reading; (8) effect of ambiant temperature; (9) effect of temperature gradients. The methods of determination and final formulae were previously given. None of these errors is greater than a few units of the sixth decimal place, most of them are of the order of $10^{-6}n$. A knowledge of them in white and in monochromatic light is required in order to determine the range, the optimum cell thickness and the light spectral purity needed to attain a stated precision under given experimental conditions. The overall effect of all the factors considered may be determined once and for all for a given instrument. Next, the final error is computed either as a formula or in tabular form, so that the ultimate operations are extremely simple, and all the involved calculations required to develop the theory of the instrument are eliminated from the computation and interpretation of the final results.

Applications and Newer Developments

Today the applications of interferometry range from the measurement of the diameter of distant suns, the plotting of star orbits, the comparison of distances, the measurement of wave lengths, the determination of the temperature of white-hot rocket gases, down to the estimation of retinal acuity, the study of electronic vibrations at the zero-energy temperature point, and the observation of microscopic phases too similar to be seen with an ordinary microscope. Most applications listed are well known in the specialized fields. A few of them, however, whose workability is facilitated by the features of the new instrument may be detailed here.

Comparison of Light Sources. The "maximum interference number" N obtainable with a given source is related to the spectral width $d\lambda$ of the line utilized by $N = \lambda/d\lambda$, and to the source temperature T by $N = k\sqrt{M/T}$, where M is the atomic weight of the element excited, or a small multiple of it. Thus either M or T may be determined as a function of one another. The comparison of sources is usually done by the tedious method of successive extinctions of the fringes. The fact that the field of view of our instrument contains an image of the entrance aperture may be used to facilitate greatly such comparisons. For the purpose, the field is divided by a small prism, each half receiving the light of one of the sources. If the two sources are not identical with respect to composition and temperature, one set of fringes moves faster than the other for the same rotation of the compensator. By observing the difference in travel produced over a rotation corresponding to some 100 or 150 fringes quite small spectral shifts may be detected. The method is akin to a sort of optical vernier, and it is, in addition, quite convenient for comparing sources which are not monochromatic.

Metrological Applications. These are made possible by increasing the working space of the instrument by means of a prism system previously described.[23] Thus rather long distances can be appraised and compared and small rotations may be measured. One early such application dealt with the measurement of the very small deflection

of a magnetic susceptibility balance.[38] Most metrological applications are amenable to study with the prism described (FIGURE 2).

Gas Analysis. The absorption of the shorter wave lengths by gases produces band spectra due to the superimposition of the effects of vibration and rotation of the dipoles. The refraction effect due to rotation becomes preponderant only in the immediate vicinity of a band. Since most gases are colorless, the refraction in the visible range involves relatively simple measurements of an additive property, and the refractive dispersion remains linear and small. Considerations of wave mechanics (Debye[14]) show that even for polar gases, contrary to the behavior of liquids, the dielectric constant generally increases at increasing frequencies, meaning that anomalous dispersion is reduced or even canceled, a circumstance favorable to such measurements. On a strictly experimental basis, the refraction of gases in visible light remains additive within the limits of error of the interferometric method. Analytical procedure for binary mixtures based on the above observations is well known. Interesting applications to metal–gas equilibria of industrial importance were reported by Le Chatelier, and by Chaudron.[10] In many contemporary applications of the method, only one gas tube is used, the comparison being made against air. In such cases, a correction must be introduced for the partial pressure m of water vapor ($\pm 0.000041\ m/760$), although the refractivity of air is *not* exactly proportional to its density. The method was more recently adapted to the study of NO_2-air anesthesia mixtures by Bell and Krantz,[2] so that flowing gases of three and even four components such as encountered in respiration studies can be analyzed almost instantly, without chemical absorption and without involved calculations. A further extension of the usefulness of the method along these lines was reported by Thompson.[51] For these analyses, the new interferometer is equipped with its deflection prism and the gas tubes are placed laterally. Thus, it offers the advantages of a small, rugged, portable, self-contained instrument capable of accommodating gas tubes of variable length. The bulk is reduced, for the gas tubes are traversed twice by the light. Several electronic devices considered in a later section will subsequently make this instrument readily adaptable to continuous recording and monitoring of a number of industrial, biological, and medical operations amenable to gas analysis.

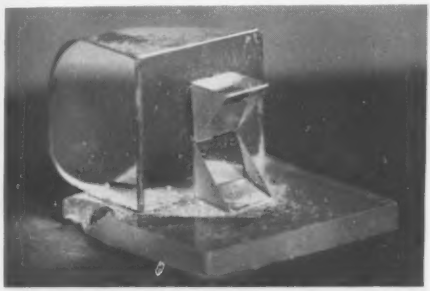

FIGURE 2. Deflecting prism for metrological applications.

The Empirical Analysis of Liquids. These may be developed on a purely experimental basis, or by the applications of the well known formulae for molecular refractivities and dispersion derived from the Lorenz and Lorentz equations. In the first case, one simply establishes experimentally the relationship between relative refractive index variations and the progress of the chemical process under investigation or a concentration variation. An example is the titration of weak polyvalent electrolytes in dilute solution in absence of an indicator. An extension of this method concerns the titrimetry in dilute non-aqueous non-conducting solutions. For instance, dilute solutions of aniline in absolute methanol are readily titrated with 0.01N NaOH in the same solvent. The unambiguous end point is found at the intersection of two very steep sections of curves comprising the plot of $n - 1$ *vs.* volume of titrant. A variant includes the continuous addition of a more concentrated titrant to a large volume of test solution so that the final volume at each step may be regarded as constant, and following continuously the index variations produced by sending a small sampling stream into a circulation cell (*v.i.*) by means of a small air-lift. Refractometry in non-aqueous solutions is often as accurate and usually more sensitive than HF titrimetry (Q-analysis). It applies best in the absence of solvent interaction, when the solvent is a nearly perfect non-polar dielectric. However, polar solvents are no contra-indications, as shown, for example, by the ease of titration of dilute aqueous phosphoric acid by this method.

Many biological problems are amenable to this experimental approach. This is the case of the

interactions in antigen-antibody systems.[32, 33, 34, 35] A $1:10^{-5}$ dilution of *Pneumococcus* Type III polysaccharide still gives a measurable *dn* at the saturation point with the specific anti-serum, while it yields no detectable precipitate. Other immunological applications previously reported[33, 34] dealt with more complex systems, certain components of which could be detected only by rather involved biological assays. Some attempts at detecting anti-hormones by interferometry are also reported in the literature.[36] The observations made along these lines are the basis of a proposed clinical diagnostic method meeting with some favor in Europe.[5, 6, 30]

Similarly, the rapid clinical determination of total serum proteins by refractometry is established on a solid experimental basis. It has been proved experimentally that serum refractivity variations are due essentially to protein concentration changes, the fluctuations in the other constituents producing a negligible effect. The temperature coefficient of the serum proteins, moreover, is negligible between 14°C. and 30°C., while that of the other constituents is negative and small, so that any positive temperature effect can be ascribed to the presence of water whose concentration, being large, can be considered constant. It follows that the protein concentration C is obtained from a single measurement of the refractivity n of the serum and the temperature t, applying a rather simple formula.[23] The refractivity increment k, per gm. of protein is determined experimentally, once and for all, for instance by Kjeldahl analysis using our method.[28] The value of k is comprised between 0.00179 and 0.00194 with an average of 0.184 (Paic and Deutsch[45]). The exquisite sensitivity of the interferometer, however, requires a re-investigation of certain aspects of the protein refractivity. Some of our data[29, 31] seem to indicate that the factor k may vary in disease. There is also some evidence pointing to a systematic variation of k with the concentration when C is smaller than a certain critical value probably comprised between 10 and 20 per cent (dilution of serum per volume). An answer to these questions is important also in connection with the interpretation of protein refractivity gradient measurements in flowing or migrating systems (*v.i.*).

Another fruitful field of empirical investigation concerns the binding power of blood proteins for various ions and radicals. Dye binding is a quite evident phenomenon of limited clinical value. Heavy metal binding, because of its narrow specificity for certain functional groups, has long found analytical applications. Binding of small ions (Na, K, Rb, F, I, etc.), however, can be readily demonstrated by interferometry[26, 27, 29] without the necessity of extracting the proteins from their natural medium. Similar but more limited experimental evidence was brought forth in the case of other substances of biological interest (ascorbic acid[37, 35] cholesterol[24, 25]). The sites of protons binding in proteins fall into six well defined groups. In general, however, the total number of sites occupied by small ions does *not* correspond to the number of recognizable radicals in a given molecule, perhaps because the procedures required to estimate the radicals are so drastic that the substance analyzed only remotely resembles the nature protein.

Other empirical applications deal with the voluminous body of work pertaining to the Abderhalden ferments.[1] The Abderhalden phenomenon is real, and the chief reason for its lack of favor among experimenters is probably that it still lacks a satisfactory explanation. Empirical interferometry, in general, is well adapted to the study of enzymic reactions, their end-products, their intermediate steps and their over-all kinetics. For instance, during the amylolytic digestion of Cl-activated starch, relatively large *dn* are registered, while an elementary theory indicates that, for the concentrations realizable in practice, about 1.5 per cent, the refractivity change should be negligible. Automatic recording methods are invaluable in these studies.

Measurements on Streaming Fluids. The analysis of streaming fluids of homogenous composition presents no difficulty. If the composition changes expected are slow, it is sufficient to analyze a small derived stream which is made to circulate, by appropriate mechanical means, into a conveniently designed cell (FIGURE 3). For these applications, automatic pointing or recording, possibly coupled with a servo-control mechanism, is highly desirable (*v.i.*).

Greater difficulties are met when composition changes occur in limited regions of a fluid moving unidirectionally, as for example, in chromatographic effluents. Then a frontal analysis is required. Ideally, it is desired to analyze an extremely thin section of the moving fluid column. The results are then referred either to the time elapsed or the distance traveled. In practice, this condition cannot be satisfied exactly, for a frontal composition gradient does not remain sharp, but,

in a moving fluid, it spreads farther than it would by simple diffusion. Thus, the instrumental requirements for such types of analysis are rather stringent (Kegeles[40]). Prismatic refractometers of the Ferry type have been used for this purpose in industry, because of the simplicity of recording they afford (Cruikshank and Fairweather,[13] and others). These instruments suffer from a lack of linearity in their response curve, undesirable dispersion effects, and absence of provision for correcting these effects. Blurring of the field occurs if layering takes place when a boundary traverses the apparatus. Definite improvement was registered by Kegeles,[39] combining a 83° prism with a schlieren system, but the utilization of the data require extensive calculations.

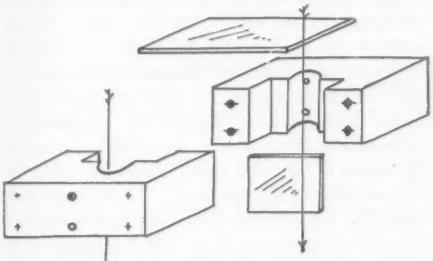

FIGURE 3. Cell for streaming fluids.

Special interferometers were previously built for the study of streaming fluids by Tiselius and Cleasson,[50] Cleasson,[11] Holman and Hagdahl,[19] Kegeles,[41, 42] Svenson,[49] Gibert,[17] Calvet and Chevalerias,[8] Calvet,[9] Coulson, Cox, Ogston, and Philpot,[12] and others. All are bulky and expensive. FIGURE 3 shows a blown-up view of an all-glass cell designed for this kind of work, to be used conjointly with the new interferometer. With this design, which embodies all the advantages of our original differential cell, the fluid streams normally to the windows. By keeping the volume small (about 0.3 ml.), satisfactory boundary compression is obtained. If a finer boundary definition is desired, one may place a narrow horizontal slit in the plane of the cell. Thus, one records the position of the fringes produced by light passing almost exclusively at the level of this slit. The accompanying loss of intensity is not very large. It is to be further noted that the field diaphragm contained in the eye piece limits the observation to a section of the cell still smaller than that delineated by the horizontal slit mentioned. In this fashion, the instrument functions similarly to that of Svenson's and that of Kegeles's in which the conjugate scanning slit in the absence of a wedge nullify the role of the second schlieren lens; for, in these instruments too, sharp fringes could be obtained without exact focussing of the camera lens onto the plan of the cell.

Diffusion Measurements. Bruins[7] previously adapted the Haber-Lowe (Zeiss) interferometer to diffusion measurements. In his arrangement, diffusion took place against a slowly moving film of near-infinite dilution. The special cell constructed for this purpose suffered from all the defects of the Zeiss cell[28] plus others introduced by the separating wedge. This wedge made it impossible to measure exactly the physical thickness of the cell and a fictive thickness had to be determined experimentally. A diffusion cell adapted to our interferometer is shown in FIGURE 4. The channels drilled in the glass plates are cylindrical. External connections are made by means of hypodermic needles cemented to the glass. The upper spacer is 0.2 mm. thick. Thus, at the level of this spacer, a very thin solvent film is produced and made to move without turbulence. Diffusion takes place in this film as in Bruins's experiments. The effective cell thickness is 10 mm. while the width of the diffusion column is reduced to 4 mm. The bottom recess is very small (2 × 0.5 mm.) and the experiment must be terminated before its content is disturbed by the effect of diffusion. In practice, it is found that the effective thickness of the cell does not vary during an experiment and that it does not depend on the initial level established in the cell. Moreover, at zero velocity,

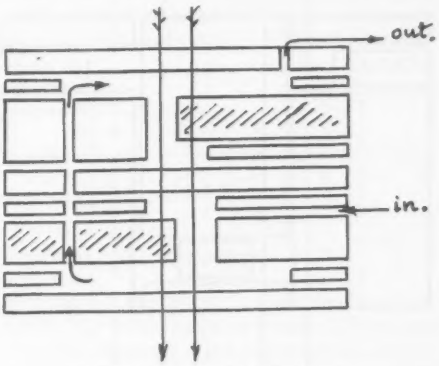

FIGURE 4. Interferometric diffusion cell.

the initial zero reading is very stable regardless of any mixing that may occur between the fluids at the begining of an experiment. Provided the diffusing substance has a refraction increment varying linearly with its concentration, the diffusion constant is given by the usual relation $\left(\frac{n_2 - n_2}{n_s}\right)^2 = 4Dt/\pi\iota^2$ (where ι is the length of the diffusing column). Diffusion against a stationary layer is best performed in the type of cell designed for electrophoretic transport observation.

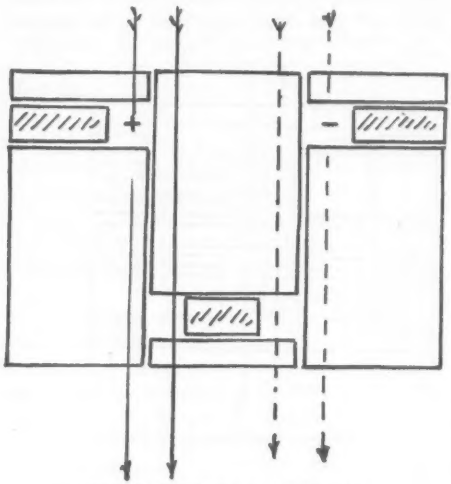

FIGURE 5. Electrophoretic cell, cross section.

Electrophoresis Studies. The problem of scanning a tall fluid column for evidence of refraction gradients, as in electrophoresis work, is quite general. The choice of the optical system depends on the acceptable minimum critical dimensions of the container where the phenomenon takes place (electrophoretic cell for instance). Detailed studies have shown that protein transference is preferably determined in a cell at least 35 mm. tall and more than 1.5 mm. wide, to avoid capillarity effects against the cell walls. Moreover, the optical path required for interferometric measurements should not be shorter than 5 mm. A discussion of the other instrumental problems involved covers the ground already explored in the study of streaming fluids. Adaptation of our interferometer to such problems was possible because the decolimating lens plays the role of the second schlieren lens in a conventional electrophoretic bench. The fringe pattern and its position is independent of the structure and position of any opaque obstruction placed between this lens and the colimator. The problem is thus reduced to designing a suitable electrophoretic cell, and to conveniently illuminating this cell by means of the interferometer colimator. A slit 20 mm. long conjugate to a lens of 32 mm. diameter and 45 mm. focal distance, satisfactorily illuminates a cell 40 mm. long. In order to keep the advantages of our differential cell design, an electrophoretic cell was built whose middle section plan is shown in FIGURE 5. The top (electrode connection) and bottom (charging channel) sections are of the conventional sliding type, and are not represented. The channels are 40 mm. tall, 5 mm. thick and 3 mm. wide so that no distortion of the fringes are produced by the vicinity of the walls. Thicker cells to be used conjointly with the deflecting prism described for metrological applications will be under study presently. It seems that with this latter arrangement, effective cell thicknesses of the order of 20 to 30 mm. are feasible (actual cell thickness of 10 to 15 mm.), since the cell is traversed twice by the light rays. At this point, three solutions are available for scanning the height of the cell: (1) one may place a slit diaphragm on the microscope stage and, without modifying the interferometer, move the cell along its vertical axis; (2) one can adopt the solution employed in the Lotmar (Kern) electrophoretic device, or the Boskamp instrument; (3) one can keep the cell stationary and scan it optically. After numerous trials, this last solution was adopted. For this purpose the microscope draw-tube was lengthened and a small cylindro-convex lens was inserted about 12 mm. behind the back-focus point of the decolimating lens, with its axis horizontal. This cylinder lens forms a virtual image of the fringes in the plan of the electrophoretic cell. A photographic lens of appropriate size (f/3.5) replacing the usual eye-piece produces a real image of both the fringes and of the cell on a ground glass placed at the upper end of the draw-tube. Every point in a direction parallel to the fringes on the image plane is conjugate to a point located on the vertical axis of the cell. Thus the shape of the fringes reflects the vertical distribution of refractivity along the cell axis, and this pattern could be photographed as such. The measurements are greatly facilitated, however, by means of the compensator used conjointly with a narrow horizontal movable slit placed in the plane of the cell. This slit can

be slowly displaced along the vertical axis of the cell and it is at all times conjugate to a corresponding level in the image-plane. Its presence results only in a loss of light intensity, but it does not affect the coherence of light passing through it. The optical diagram is shown in FIGURE 6. If this slit is sufficiently narrow (about 0.3 mm.) the fringes observed are straight again. Thus, the lateral displacement of the fringes measured by the compensator, is a measure of the difference in refractive index between the fluid in the reference cell compartment and that in the active limb, at a level whose height is exactly measurable on the final image observed. The observations are facilitated by a small magnifier focussed on the ground glass mentioned, after adjustment this glass can be entirely removed. Plotting of the ob-

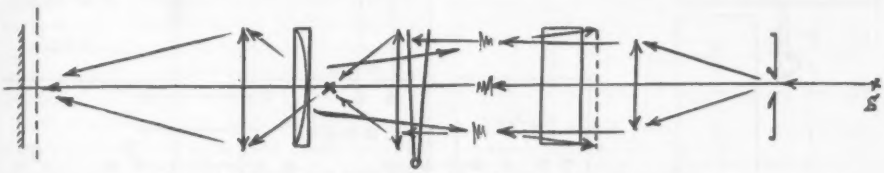

FIGURE 6. Microscope interferometer for electrophoretic studies, optical pathway.

served relative *dn versus* cell height thus takes only a few minutes. Both ascending and descending limbs can be explored in the same fashion. The problem of automatic recording, plotting and monitoring, other than by photography and very desirable for this type of work, is considered in the next section.

Instrumentation for Refractive Dispersion Measurements and Recording. The key to the interpretation of refractive index measurements in terms of molecular structures may be found in the formal similarity discovered by Hamilton, between de Fermat's theorem and Maupertuis' intuitive principle of "least action". The latter states that the trajectories of a material point in motion are always such that the sum of the energies, U and E, in minimum $\delta \int \sqrt{U + E}\, ds = 0$. Thus, in steady field derived from a potential function $U(x, y, z)$, the trajectory is such that the corresponding integral $\int \sqrt{2m(E + U)}$ is stationary. The similarity with de Fermat's principle appears when one postulates $\sqrt{U + E} = n$. In this way, the refractive index of a medium expresses the force function existing in it which determines the trajectory of the electromagnetic energy.

The great amount of experimental work stimulated by the theories of Young-Fresnel and that of Maxwell, revealed that in fact the refractive dispersion of transparent substances is governed by their ability to absorb the radiations. The quantitative relationships existing between these two quantities, however, are complex, and the passage from Cauchy formula (for substances with narrow bands in the *UV*) to the more general formula of Maxwell and of Sellmeyer, or that of Helmholtz-Ketteler, involves a number of simplifying assumptions. That contemporary quantum theory and wave mechanics calculations, generally substantiate these assumptions does not remove their indeterminate character which stems mostly from the fact that refractive index measurements outside the visible spectrum remain difficult and relatively inaccurate. The great practical interest of such measurements is found in the observations and theories relating the refractive index to the dielectric constant in the radio-range of wavelengths, to the London vibrational energy of dipoles at the zero energy point, and to a host of other phenomena related to the polarization and/or polarizability of molecules under the influence of the electromagnetic field. That the calculations involved in such studies are sufficiently intricate to place them outside the reach of the average chemical laboratory not specializing in these questions, makes it still more desirable to develop an instrumentation capable of gathering the desired data in an empirical, if exact fashion. For, at the chemical standpoint, "anomalous dispersion" is the rule for all substances in the vicinity of an absorption band anywhere in the spectrum, and its determination as a measure of the "chemical exaltation" (of refractivity) acquires a meaning complementary to that of spectrophotometric absorption.

An attempt was made in this laboratory to adapt the new interferometer to dispersion measure-

ments over an extended range of frequencies. The problem of observing a shift of interference fringes outside of the visible spectrum amounts to (1) finding suitable transparent optical parts (2) finding suitable sources of radiations (3) finding suitable radiation detectors and (4) finding suitable means of "reading" the "field of view." The first, second, and third of these desiderata, have been solved with contemporary advances in the field of *UV* and *IR* spectrophotometry. The optical requirements of the interferometer are not terferometer, without cells, was adjusted in white light (5890 *uÅ*). The central achromatic fringe can thus be easily spotted, by opening, to its fullest extent, the monochromator slit. Two pending projects are in progress at present, one will involve replacing the interferometer lenses with optics made of arsenic trioxide glass, the other involves the design of suitable reflecting optics of the type developed by Blout for infra-red microspectrophotometry. Pending these developments, the instrument eye piece was removed, and the

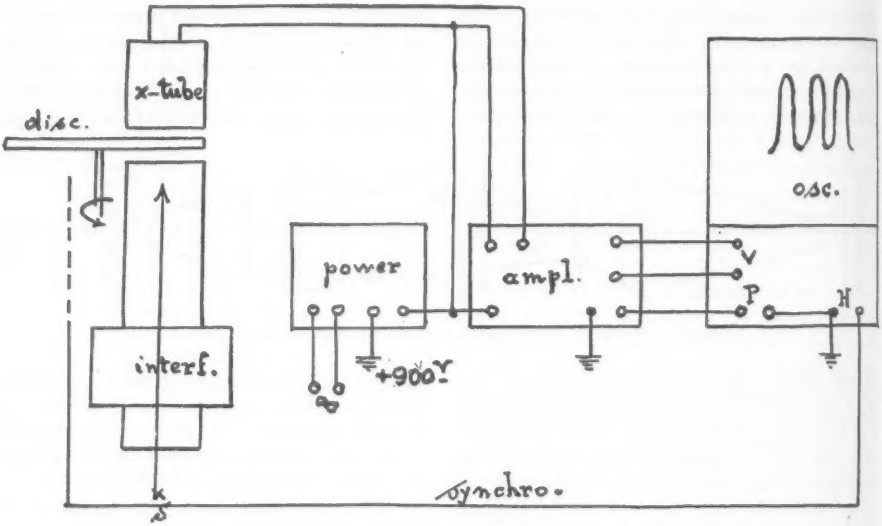

FIGURE 7. Block diagram of interferometer recorder.

stringent, and the number of parts is limited to three, or even two, lenses and a few flat plates for the construction of the cells. There remains, however, one restriction namely that no single solution of these desiderata covers adequately the whole range of radiations. The following summarizes our efforts at solving the fourth set of requirements.

In order to produce a "non-visible" interference pattern for preliminary study, the apparatus was equipped with a quartz colimating lens, and was adjusted to receive the light transmitted by a model *DU* quartz Beckman spectrophotometer used as a monochromator. The normal lamp housing was removed, and a 6 v. 120 w. projection bulb rated at 6.4 v. was used in its place, for both *UV* and *IR* studies, the light being concentrated by a suitable front-aluminized mirror. The in-draw-tube length was increased to 2½ feet, thus ensuring the recombination of the light rays at this distance without additional optics. The nature of the radiation detector used in these studies is irrelevant to the demonstration sought. Some preliminary studies were conducted, therefore, with one of each type CV-147, and CRI photo-converter tubes ("Bildwandler"). These tubes require only 6000 v. for their excitation. They gave quite satisfactory image transcription of the interference pattern in the near *UV* and the near *IR* ranges, but they became rapidly "fatigued" when exposed to visible light, so that they offer little help during the preliminary adjustments of the apparatus. Therefore, photomultiplier tubes—eventually with especially sensitized cathodes—working at low temperature appear more promising although the accessory instrumen-

tation required is somewhat more involved, and in subsequent studies a 931-A tube (RCA) was utilized.* With this tube, an adaptation of the Nipkow disc was utilized as a means of "reading" the shape and position of the interference pattern. The disc bore 42 slits, each 0.2 mm. wide. The rotation speed was 1800 rpm. Thus the frequency of light interruptions was 156 cps, a non-harmonic number of 60. The disc was interposed between the interferometer image-plane and the 931-A tube. The output of the tube was fed to a Bogen sound amplifier, as shown on the block diagram, FIGURE 7. This arrangement obviated the need for a DC amplifier, had slower light interruptions been produced, and the Bogen amplifier gave a very satisfactory output signal. This signal was fed to the vertical plates of a 6 inch cathodic oscilloscope whose horizontal plates were properly synchronized. Thus the entire interference pattern was translated on the oscilloscope screen with sufficient clarity to allow exact pointing of the positions of the fringes.

While it is premature to anticipate the value of data secured with the equipment described, it is worth reporting that good fringes were obtained with the 931-A tube in the range 3800 to 11000 μA, and that a satisfactory compensation remained possible, within ⅛ of one fringe in the entire range. Thus it appears that a further extension of the range of dispersion measurements possible with the interferometer will depend primarily on the choice of the optics and of the radiation detectors available.

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* The 900 volts power supply block required by tube 931-A was donated by N. A. Philips Company, Export Division, Mount Vernon, New York, whose cooperation is hereby gratefully acknowledged.

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SECTION OF BIOLOGY

INFRARED MICROSPECTROSCOPY—INSTRUMENTATION AND SOME BIOLOGICAL APPLICATIONS*

By ELKAN R. BLOUT†

Although during the last ten years it has become increasingly evident that the infrared absorption spectrum of an organic compound is the most nearly unique physical chemical property of that compound, the application of this powerful tool to problems of biology and biological chemistry is only now being made. Since biologists and biochemists often work with minute quantities of material, either in the form of tissues or products extracted from living organisms, techniques for identification and characterization of such materials in very small quantities prove useful. Infrared microspectroscopy is such a method.

Microspectroscopy itself is not new. The first microspectroscope was described by Sorby in 1865[1] soon after the invention of the spectroscope. This was a microspectroscope for use with visible light and was applied to the detection and characterization of blood stains. About forty years later Köhler[2] invented an ultraviolet microscope and Caspersson in the 1930's combined this microscope with a spectrometer and studied the ultraviolet absorption of many different types of tissues.[3] However, it was not until the last few years that infrared microspectroscopes have been designed, constructed and used, the principal work having been reported from laboratories in this country[4, 5, 6, 7, 8] and in England.[9, 10]

Several characteristics of infrared microspectroscopy have been considered. An infrared microspectroscope is essentially a combination of a spectrometer and a microscope which is achromatized over the large spectral region between 1 and 15 microns (10,000 to 666 cm^{-1}). The microscope may be placed between the radiation source and the spectrometer and the enlarged image of the specimen is then focused on the entrance slit of the spectrometer. Alternatively, the microscope could be placed after the exit slit of the spectrometer and the specimen imaged onto the thermal detecting element. In either case, however, the magnification of the microscope should be low, since no gain in performance can be obtained from having magnifications higher than about 15 times. This is true because the useful magnification, that is, that magnification which gives the optimum performance, is equal to the numerical aperture of the microscope divided by the numerical aperture of the spectrometer.[4] Since most infrared spectrometers have a numerical aperture of approximately 0.1, it is not necessary to go beyond about 15 times magnification. Secondly, since the wavelength of infrared radiation is much longer than that of visible radiation, it would seem that it is not possible to resolve objects as small as is possible in the visible and ultraviolet. In fact, from diffraction theory, it is apparent that, for work at wavelengths as long as 15 microns, the object to be measured must have at least two dimensions of 9 microns or greater. Thirdly, since the beam of infrared radiation will enter the sample through a wide range of angles, if high numerical aperture microscope objectives are used, it may be necessary for quantitative work to correct the absorption actually recorded to take into account the different pathlengths through the specimen. This correction has been described[4] and appears to be significant only for quantitative work at numerical apertures greater than one. Finally, we have suggested a figure of merit of performance which characterizes an infrared microspectrometer.[4] In general, the performance of an infrared spectrometer is dependent on: (a) the spectral resolving power; (b) the time constant of the system, that is, the time necessary to receive the information; (c) the area of the specimen; and

* This paper, the first of three papers in a symposium on Recent Advances in Cytochemistry, was presented at a meeting of the Section on May 11, 1953.

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(d) the signal-to-noise ratio of the detector-recorder combination.

With an infrared microspectrometer now available, it is possible to obtain an absorption spectrum of an organic compound with good resolution using only one to 10 micrograms of material, which is approximately one-tenth to one-thousandth the quantity of material required by conventional infrared macrospectrophotometric techniques. The sample may be measured as a deposit on an infrared transparent support, as a crystal, or as a solution contained in a special type of microcapillary cell made of silver chloride. Samples of the first type include not only pure chemical compounds, but tissue extracts and tissue sections.

Work on the infrared spectra of tissues has indicated that many tissues have very similar infrared spectra which are reminiscent of the spectra of proteins. Certain tissues, however, show differences due to the presence of large amounts of lipids or nucleic acids. It would appear, however, that unless by some ingenious method one could increase the resolving power of the infrared microspectroscope so that the spectra of parts of cells such as nuclei, nucleoli, *etc.* could be determined, the most fruitful work will be done on the identification of extracts of tissues from various organs and perhaps on the characterization of the extracted tissues.

Recent results indicate that extracts of different tissues yield quite different infrared spectra and work is now under way to isolate and identify the chemical components by infrared microspectroscopy. In this connection it should be noted that paper chromatographic separation and infrared microspectroscopy offer methods which are mutually complementary. Paper chromatograms usually are run so as to deposit 1 to 10 micrograms in each "spot." This is precisely the right order of magnitude for infrared microspectroscopy and it would appear that the extraction of such "spots" with subsequent determination of the infrared spectra of the solute should prove to be a useful method for the separation and identification of microgram quantities of organic compounds.

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RESPIRATORY STUDIES ON SINGLE CELLS—METHODS, INSTRUMENTATION, OBSERVATIONS*

By C. LLOYD CLAFF†

Any discussion of micro-respiration must acknowledge the magnificent contributions made, over a long period of time, by Linderström-Lang, Holter, and their coworkers[2, 4, 5] at the Carlsberg Laboratory. Therefore, as an introduction to the micro-micro-liter reference diver method reported in 1952 by Scholander, Claff and Sveinsson,[9, 10] I would like briefly to touch on the Cartesian Diver method, and some modifications of it, which I have made to extend and enlarge its usefulness.

In tracing the history of any method, it is interesting to see how far back you can push the references. We find that Raffaele Magiotti,[1] a pupil of Galileo, in 1648 published the first account of the so called "Cartesian Diver." Magiotti wrote one paper on the *in*compressibility of water, and the compressibility of air, with a figure of the Cartesian Diver. The book is in the British Museum. I have had it micro-filmed and translated by Hannah Crosdale. It is an exceedingly interesting paper, not only because of its reference to the Cartesian Diver, but also because it gives us an insight into the mind of a seventeenth century scientist. Let me quote the opening paragraph of this paper, which is entitled:

* This paper, the second of three papers in a symposium on Recent Advances in Cytochemistry, was presented at a meeting of the Section on May 11, 1953.

† Research Associate in Surgery, Harvard Medical School, Boston, Mass.

VERY CERTAIN RESISTANCE OF WATER TO COMPRESSION EXPLAINED WITH VARIOUS TRICKS IN OCCASION OF OTHER CURIOUS PROBLEMS

AT ROME. Through Francesca Moneta 1648
With Permission of the Superiors
To the Most Serene Prince
DON LORENZO MEDICI

"Wrongly, (Most Serene Prince) have certain people grieved about being born too late, when the secrets of the universe have all been dis-

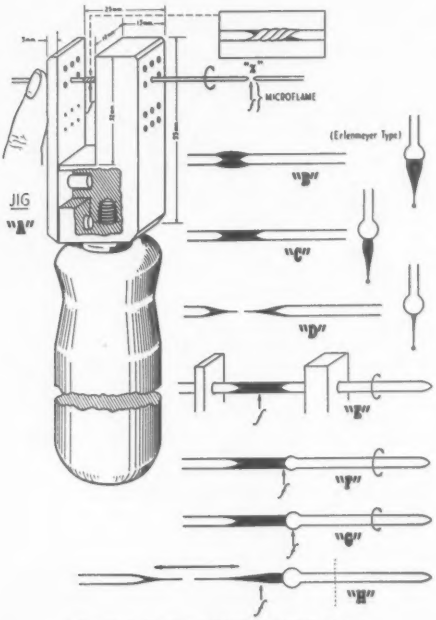


FIGURE 1. Cartesian Diver Jig (Claff, 1948).

covered; because, although the world should last more years than were the days of Nester or Noah, the mine of inventions and the stupendous works of nature would never be exhausted. On the contrary, those that remain for posterity are the more beautiful and more marvelous as they are the more hidden and more difficult. Such people answer that this remainder is none other than that Nature, so as not to stand in idleness, produces some little thing new. But these are mere illusions; since the little valves of our veins and arteries, the circulation of the blood, the palpitation of the heart are as old as Adam himself; and speculation is as new as Harvey, the finest intelligence of our century. Thus this resistance to compression which I describe (be it said without arrogance) is as old as the creation of water, and as new as its explanation is totally contrary to common opinion. Receive, Your Highness, with your customary benignity this early fruit of mine, though it is unripe indeed and still with its first down; and be indulgent to my fear that an invidious hand might pluck it."

In 1937, nearly 300 laters later, there appeared in "Nature" a short article by Linderström-Lang,[5] on the theory of the Cartesian Diver, suggesting

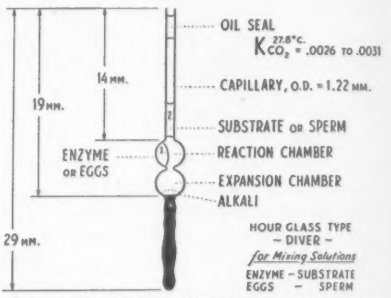


FIGURE 2. Double bubble Cartesian Diver (Claff, 1949).

its use as a scientific instrument for gasometric analysis and respiration studies. Two years later, in 1939, Boell, Needham, and Rogers[3] published an article in the "Proceedings of the Royal Society" in which they described their use of this instrument for measurements of oxygen consumption.

I reported in a series of papers from 1947 to 1949[6, 7, 8] refinements for ease of operation, and instrumentation for extending this method so that "mixing" could be accomplished by the use of a double bubble in the diver (FIGURES 1 and 2).

It is possible to follow morphological changes and observed behavior of material through the microscope of the bath of the Cartesian Diver, and correlate these with the respiration obtained. Sonneborn brought to light the fact that *Paramecium* could be typed according to "mating." *Paramecium calkinsi*, types I and II, illustrate "mixing" technique and correlation of observed behavior and respiration, (FIGURE 3). A more involved "mixing" technique is shown in FIGURE 4.

There have appeared other modifications of the

Cartesian Diver method, notably those of Eric Zeuthen, working at the Carlsberg Laboratory. He drastically reduced the size of the diver, and improved the sensitivity of the water manometer. In discussing some of the results of our own experiments with the micro-micro-liter reference diver, we will return to Zeuthen's studies and compare our results with his.

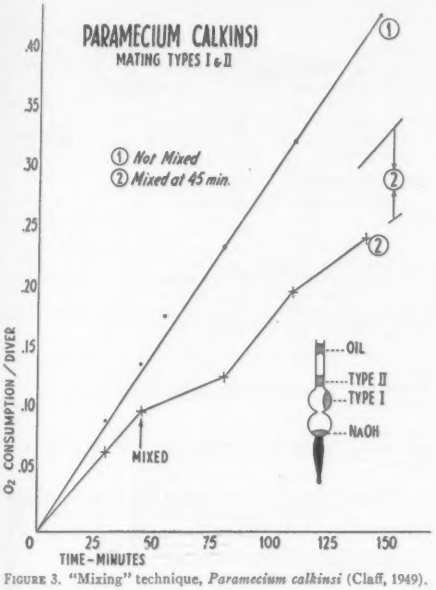


FIGURE 3. "Mixing" technique, *Paramecium calkinsi* (Claff, 1949).

In collaboration with P. F. Scholander, during a period of six months in 1949, under a grant from Harvard Medical School Milton Fund, a new technique known as the reference diver technique, or micro-micro-liter diver, was developed.

The method is, in some respects, simpler than the Cartesian Diver. It has a sensitivity of 0.2 micro-micro-liter and a stability or drift of 0 to 10 micro-micro-liters per hour. A micro-micro-liter is defined as a millionth of one cubic millimeter or a volume equal to a cube with sides 10 microns long.

In 1911, Krogh developed a method of micro *gas* analysis which consisted of measuring the diameter of gas bubbles under a microscope, before and after the absorption of CO_2 and O_2. Scholander thought we might use this method. The respiring cell and bubble of gas would be put into a small vessel, connected by a diffusion path to atmospheric pressure in the water bath. The cell would consume the bubble, and the rate of shrinkage could be measured through a microscope. Tests were made and the stability was good, but as a bubble shrinks, the *surface tension* builds up an increasing gas pressure in it (FIGURE 5).

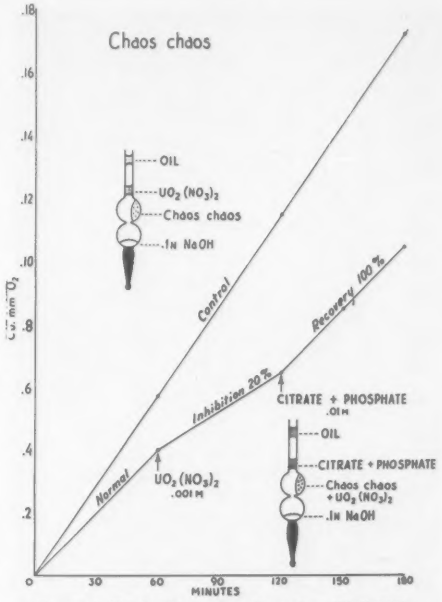


FIGURE 4. "Mixing" technique, *Chaos chaos* (Claff, 1949).

It became immediately apparent that it would be necessary to keep the bubble volume constant. In a Manometric system, if the bubble size, that is, the gas volume, and the liquid content of the chamber is kept constant and is known, then the change in pressure will be an index of the oxygen consumption. This then was our goal; to create a constant volume system, where the pressure changes, read on a mercury manometer, and multiplied by a factor calculated from known constants, would give us the oxygen consumption. The problem then was how best to know we had the bubble at a constant size, as we varied the pressure to compensate for the bubble's loss of oxygen. Two possible solutions presented themselves. The optical measurement of the diameter of the bubble, the first tried, showed in a series of observations, a variation of 3 per cent in volumes

calculated. A much more sensitive method is the flotation method. By sticking a hydrophobic minute weight on the bubble, the variation in the pressure necessary to keep it at a reference point became the index of oxygen consumption.

A piece of Saran tubing was heated over a micro flame, and pulled out to form a capillary tube, with a diameter of 40 μ and a 2 μ lumen. A small piece was selected, and attached to a suitable

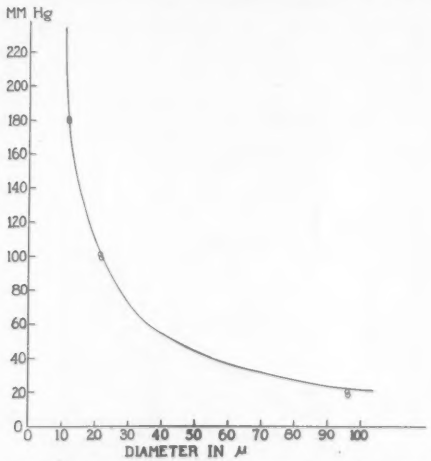


FIGURE 5. Surface tension gas pressure in bubbles. Three double checks, six points on line, show pressures obtained by Scholander, using three calibrated capillaries, and recording mercury manometer pressure necessary to form bubble just under surface of water (Scholander, unpublished).

cutting table under a microscope. Using a piece of razor blade, a 45 degree angle cut was made, and then the lumen was pressed together by a blunt razor blade, and cut off to a length approximately one and one-half times the diameter of the tubing. This formed the reference diver (FIGURE 6).

A piece of *soft* glass 3 mm tubing was heated and pulled out to a uniform taper. A glass rod was pulled out to a uniform taper and a piece selected with an end of approximately 0.3 mm. This was tried in a number of glass tapered tubes, until a colored Newton ring fit was accomplished. This is the criterion, that the liquid connection from the chamber to the outside is sufficiently narrow. The capillary was sealed off to form a chamber (FIGURE 7).

The rod was then pulled out, the chamber filled with oxygenated water, and into this we deposited the respiring material and the reference diver. The plug was reseated and the respiration chamber was dropped into a respiration tube about 4 mm in diameter.

This whole assembly was placed in a water bath, with a temperature control of $\pm.005$°C. and the tube was connected to a mercury manometer by a small polyethylene tube. The mercury manometer was connected to suction, atmospheric pressure, and to a fine pressure *device*.

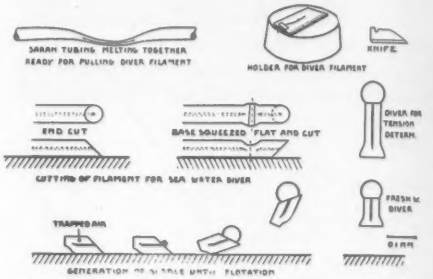


FIGURE 6. Method of forming reference bubble diver (Scholander, Claff and Sveinsson, 1952).

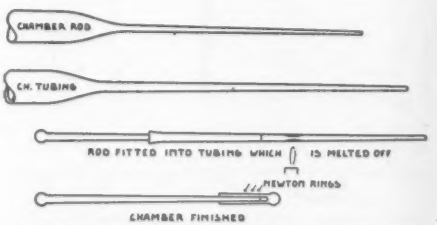


FIGURE 7. Method of forming respiration chamber (Scholander, Claff and Sveinsson, 1952).

By applying suction to the system, the air trapped in the lumen of the reference diver expanded out into a bubble, which finally became large enough to float the diver up. By means of a fine pressure device, the diver was made to come to rest at a reference point in the ocular of the bath microscope.

As the respiring material used up oxygen in the chamber, the oxygen tension of the liquid phase became negative to the gas phase in the bubble, and gas passed into the liquid phase, thus reducing the size of the bubble. The diver started to lose buoyancy and sink. Negative pressure was needed to keep the diver at the reference point. This change in pressure was the index of oxygen consumption. No alkali has been provided for CO_2 absorption because the gas phase of this

system is less than 1 per cent of the liquid phase. The liquid phase has ample ability to absorb the gas produced. There is available for the respiring material an oxygen volume equal to 3 per cent of the total liquid volume, so that the liquid volume, when RQ = 1, has to absorb, at most, 3 per cent of its volume of CO_2 (usually only about 1 per cent). The coefficient of solubility of CO_2 is thirty times that of O_2. When the liquid is acid, the largest error possible is a 3 per cent low O_2 reading. The effect of adding known amounts of CO_2 to water was tested and reported by us in 1952, and checks with the theoretical effect (FIGURES 8, 9, and 10).

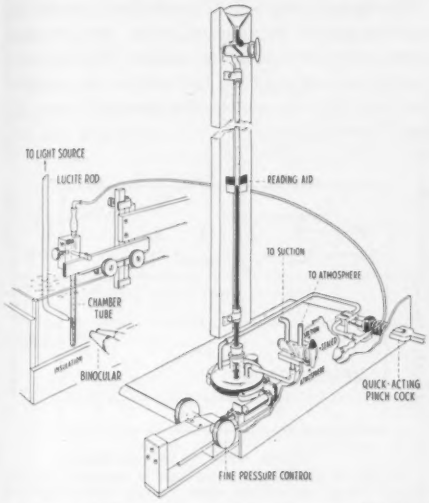


FIGURE 8. Micro-micro-liter reference diver system (Claff).

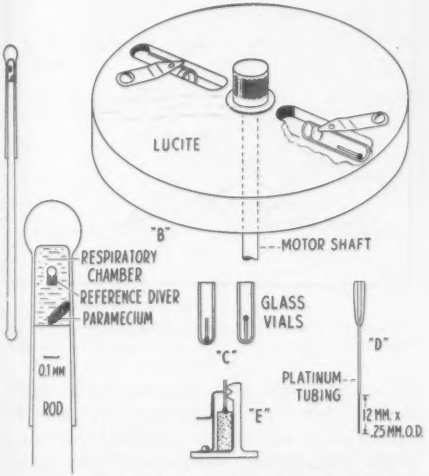


FIGURE 9. Lucite head of centrifuge for filling respiratory chamber. Method of delivering respiratory material to chamber is also shown (Claff).

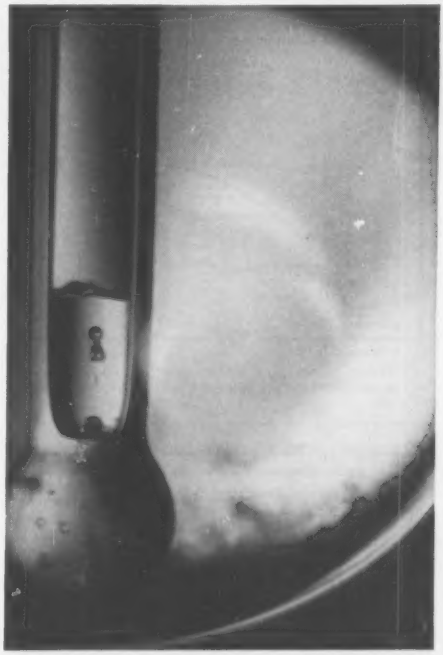


FIGURE 10. Respiratory chamber containing reference diver and respiring material (fertilized egg) as seen through microscope of water bath (Claff).

An alkali absorption system is necessary in the Cartesian Diver, because the gas phase in this system is so much larger than the liquid phase of the system.

An idea of the sensitivity of this system may be seen from respiration studies on a *Paramecium* (FIGURE 11). The chamber, in this case, was made of Pyrex glass. We found that a freshly made chamber of Pyrex glass was lethal to *Paramecium*. Here you see the diminution of respiration, the bursting of the *Paramecium*, and the surge of oxygen uptake by the released enzymes.

There is inherent in this system, an exceptionally rapid tension equilibrium. This can be determined empirically by setting up a blank run,

without respiring material, and plotting every minute, a ten minute run. By putting a 100 mm mercury overpressure on the system, the bubble is made to dissolve in the water, and the diver sinks. The diver is brought to flotation again at the reference point, and the flotation pressure will be found to be within half a mm of its former reading, within 30 seconds.

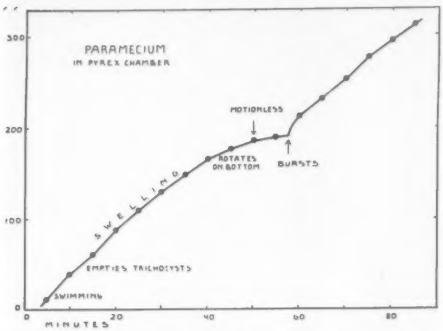


FIGURE 11. Sensitivity of system seen from quick response to *Paramecium* disintegration (Scholander, Claff and Sveinsson, unpublished).

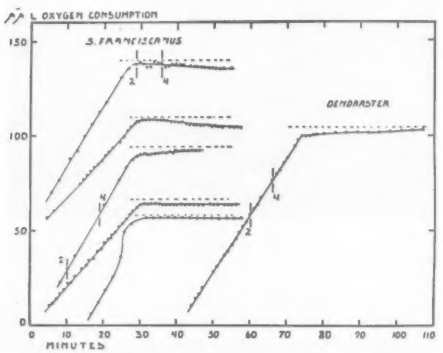


FIGURE 12. Influence of low oxygen tension on the respiration rate and on cleavage (Scholander, Claff, Sveinsson and Scholander, 1952).

Diffusion *distances* are very small in the system. Diffusion of gases through the sleeve are too small to be measured, and we have never been able to pump a colored dye from the respiratory tube into the respiratory chamber.

The temperature of the water bath must be held close to tolerances of ±.005° C. (usually taken by us on a Beckmann thermometer with every manometer reading). Also, an appreciation of the effects of bacterial contamination must be uppermost in the mind of the operator, and proper techniques used to reduce this insidious source of error.

We tested the effect of low oxygen tension on the respiration rate by charging the chamber water with *air* instead of *oxygen*. The rate remained normal until the last one or two minutes (FIGURE 12). In one of Scholander's runs, he was most fortunate to have his oxygen run out

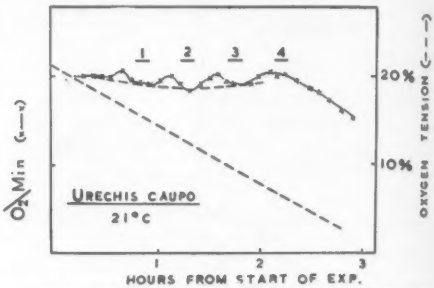


FIGURE 13. *Urechis caupo* (700 eggs in modified Cartesian Diver). Oxygen consumption shows a mitotic rhythm (Zeuthen, 1949).

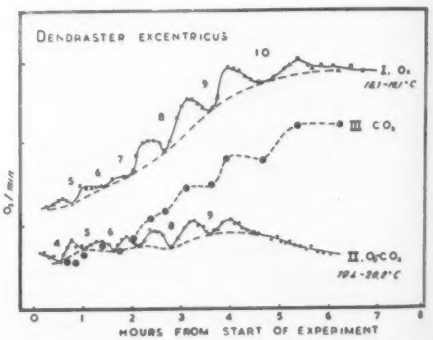


FIGURE 14. *Dendraster excentricus*, rhythmic cycling (Zeuthen, 1949).

just at the start of a cleavage furrow (upper curve, FIGURE 12). The division went on to full completion in spite of the complete cessation of oxygen consumption! Here was a direct demonstration that protoplasmic division, at least in one species, can be accomplished without *oxygen* consumption.

A drastic event, such as the division of a cell into two cells, would appear as a natural place to look for an uptake in oxygen consumption.

Many authors have reported studies of this nature with positive and negative results. Needham (1931) and Brachet (1951) have published rather complete surveys of this work.

Zeuthen (1949–50–51)[11, 12] reported cycling to be a constant occurrence in eggs of sea urchins (*Psammechinis miliaris*, *Strongylocentrotus*), a sand dollar (*Dendraster excentricus*), and a marine worm (*Urechis caupo*), which he ran in batches of two hundred to seven hundred eggs at a time, in a modified Cartesian Diver.

During the summer of 1951, we undertook to analyze oxygen consumption on single eggs of the following forms: Two sea urchins, (*Strongylocentrotus purpuratus* and *Strongylocentrotus franciscanus*); a sand dollar (*Dendraster excentricus*); two worms, (*Urechis caupo* and *Chaetopterus pergamentacous*). With two exceptions, these forms were used by Zeuthen in his mass runs. One of Zeuthen's main summary points is, "In the segmenting egg the oxygen consumption follows a rhythm, which throughout segmentation correlates with the mitosis." To support this point, we see in FIGURE 13 (Zeuthen) rhythmic fluctuation, or cycling, correlated with the first four divisions (700 eggs in a modified Cartesian Diver). This is further demonstrated by Zeuthen in FIGURES 14, and 15.

Comparing the published mass runs (FIGURES

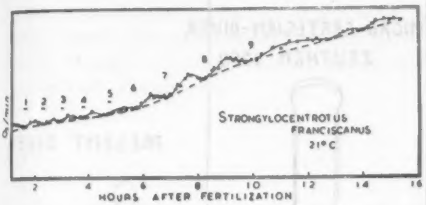


FIGURE 15. *Strongylocentrotus franciscanus*, rhythmic cycling (Zeuthen, 1949).

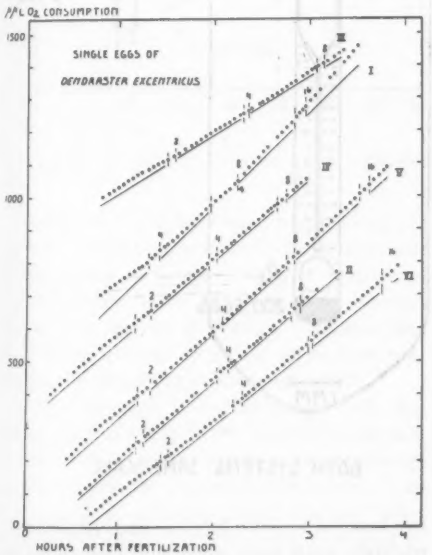


FIGURE 16. *Dendraster excentricus* (Scholander, Claff, Sveinsson and Scholander, 1952).

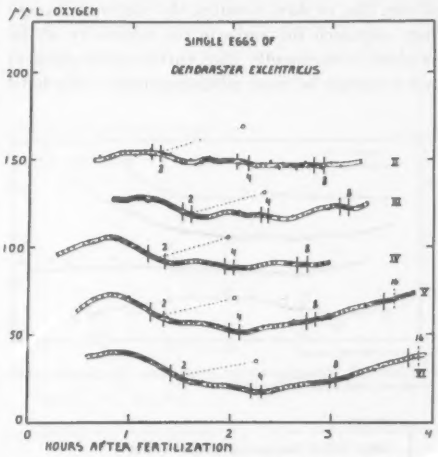


FIGURE 17. *Dendraster excentricus* (Scholander, Claff, Sveinsson and Scholander, 1952).

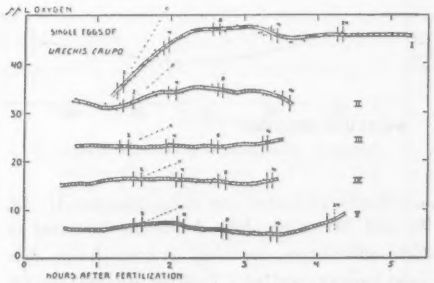


FIGURE 18. *Urechis caupo* (Scholander, Claff, Sveinsson and Scholander, 1952).

13, 14, and 15) with our own data, taken from our single fertilized egg experiments, we find some interesting discrepancies in results. We did not find cycling or rhythm to be a constant occurrence, although we did observe it in some cases. If it did occur, it disapeard after the first few divisions.

When observations are taken at short intervals of one, two, or three minutes, the observed changes may approach the limits of the sensitivity of the method, consequently rate curves from point to point cannot be used advantageously. We have

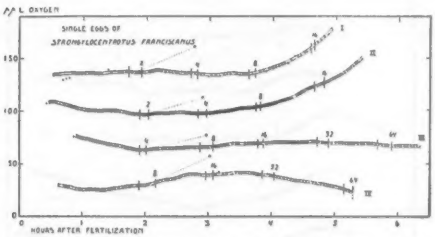


FIGURE 19. *Strongylocentrotus franciscanus* (Scholander, Claff, Sveinsson and Scholander, 1952).

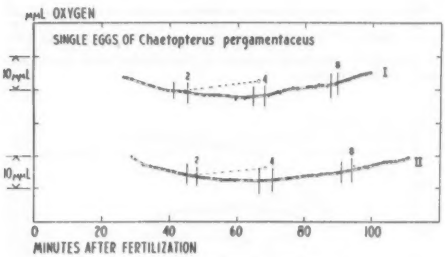


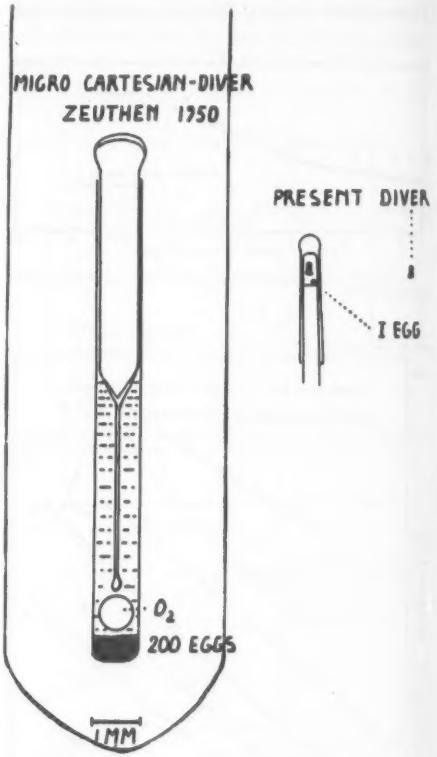
FIGURE 20. *Chaetopterus pergamentaceous* (Claff).

accordingly presented our data (FIGURES 17, 18, 19, and 20) as absolute deviations in terms of micro-micro-liters, from an average linear rate (solid lines FIGURE 16). Position of curves on the ordinates are arbitrary.

Dendraster excentricus (FIGURE 17) shows some evidence of cycling, strongly damped however, and disappearing after the third division (graphs V and VI). *Urechis caupo* (FIGURE 18) shows no cycling except in graph II.

Strongylocentrotus franciscanus (FIGURE 19) and *Chaetopterus pergamentaceous* (FIGURE 20)* likewise showed no cycling although sometimes a slight rate change at cleavage was noted.

Now let us consider the material used and the conditions under which oxygen uptake was measured. In the modified Cartesian Diver, there were



two hundred to seven hundred eggs, packed in a relatively small space where individual cleavages could not be adequately observed.

If we are to accept these periodic upsurges of respiration as correlated with cleavage, I believe we must establish as a premise: (1) a significant number of eggs of the batch used divided in a synchronous, orderly manner; (2) all cells of *single individual* eggs divided at the same time. There seems to be some anatomical evidence to the contrary. Scott and Fox,[13] working with *Arbacia*

* I wish to acknowledge technical assistance of Barbara Orski in obtaining data for this figure.

eggs, found on coordination of eggs from one female; first, a progressive loss of coordination of one egg with another after the third cleavage; second, in individual eggs, while the nuclei of the individual eggs cleaved nearly simultaneously in the second and third cleavages, thereafter the animal cells and the vegetal cells vary from five to ten minutes and, by the sixth cleavage, the difference is in excess of twenty minutes. If this is so, it would seem to me rather difficult to correlate recorded upsurges of O_2 consumption with synchronized mitosis, in mass runs.

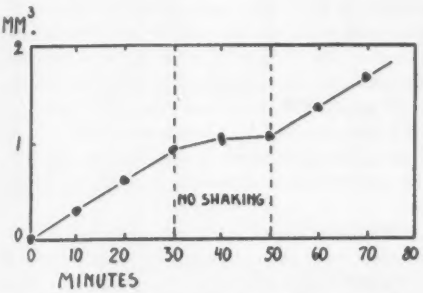


FIGURE 21. Gas tension gradient effect in yeast (Scholander, Claff, Andrews and Wallach, 1952).

When two hundred to seven hundred eggs are packed together without shaking, we must not be indifferent to the possibilities of oxygen and carbon dioxide tension gradients, both of which might effect the rate of cleavage. Evidence that tension gradient can be present under these conditions can be seen by studying FIGURE 21.

Here is a graph from Scholander, Claff, Andrews and Wallach (1952),[14] which shows that in a sensitive system, like yeast, the *absence of shaking*, in a packed mass, dropped the respiration to as low as 10 per cent of its normal rate and, when shaking was restored, the respiration returned to its normal rate.

Much valuable data has been collected by mass runs, this is appreciated by everybody. However, when it comes to isolated events characteristic of single cells, such as cell division, it seems that the single cell approach should be considered as preferable. We recognize that the ability to measure extremely small amounts of oxygen uptake has been gained at the sacrifice of versatility. Mixing and shaking have not, as yet, been incorporated in the method.

FIGURE 22 shows cell volume of single fertilized marine eggs in relation to rate of oxygen consumption. Each point represents data from a single egg. It shows that, in general, O_2 consumption of this type of material is between one hundred and three hundred micro-micro-liters per hour, under the conditions of our experiment. It is evident that there can be marked differences in the volume of eggs from the same species, and

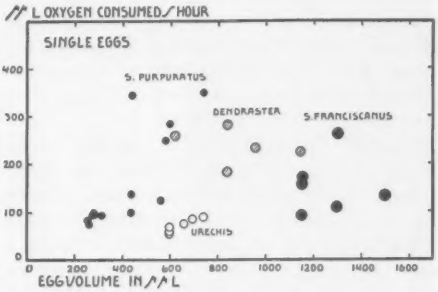


FIGURE 22. Cell volume in relation to rate of oxygen consumption (Scholander, Claff and Sveinsson, 1952).

eggs of the same volume show marked differences in rate of O_2 consumption. Much more evidence must be collected, especially from single egg runs, before conclusive evidence is established for the relationship of cell activities and respiration.

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SUBMICRO METHODS IN ENZYMATIC CYTOCHEMISTRY*

By M. J. KOPAC†

Most living cells, if supported in isopyknotic media, can be centrifuged at high speeds without flattening, so that all cytoplasmic inclusions become displaced and rearranged, in accordance with their densities, to form strata.‡ The cells will elongate with this procedure and layers of living cytoplasmic matrix, uncontaminated by subcellular particulates (nuclei, mitochondria, lipids, secretory granules, *etc.*) are obtained.

Samples of particulate-free matrix that can be extracted with a micropipette from centrifuged cells of vertebrates are necessarily small. Except for certain somatic cells of *Amphiuma* and other amphibians, most cells are so small that not much more than 1 $\mu\mu$L** of matrix (diameter of spherical volume = 12.4 μ) can be removed. At best, the largest samples that can be obtained will rarely exceed 10 $\mu\mu$L.

For these reasons, the volumetric submicromanipulator was designed and constructed.†† With this new instrument, volumes of cytoplasmic substance of the order of 1 $\mu\mu$L can be removed from a living cell and transferred to substrate mixtures.

Enough is known about the enzymatic activities of certain cells to permit estimation of the sensitivities required to measure the expected activities in 1 $\mu\mu$L of cytoplasm. For example, the alanylglycine peptidase activity in $\mu\mu$M/hour of 1 $\mu\mu$L of cytoplasm from the following cells is: *Amoeba proteus*, 3.2; *Pelomyxa carolinensis*, 4.0; unfertilized *Arbacia* eggs, 7.3; and unfertilized *Dendraster* eggs, 13.3.[1]

Obviously, to measure the activity of a portion of a cell, the matrix fraction for example, there will be required not only instruments and methods for removing a measured volume of that fraction from a cell but also methods sensitive enough to measure activities in the order of $\mu\mu$M/hour.

It has already been established that certain enzyme-substrate reactions produce volume changes. If the substrate on hydrolysis yields dipoles or ions, then such resulting electrostriction reduces the reaction volume and consequently increases the density, if mass remains constant. For example, the following constriction constants, $-\Delta V$ in $\mu L/m$M, have been reported:[1] hydrolysis of L-alanylglycine, 9.7; depolymerization and release of phosphate from desoxyribose nucleic acid, 13; and hydrolysis of phosphate esters in acid pH, 3.

Linderstrøm-Lang and Lanz[2] described a micromethod for measuring the hydrolysis of dipeptides by small amounts of enzymatic material. Their methods, based on the fall of a reaction drop (increase in density) in a density gradient column, were sufficiently sensitive to measure the splitting of 30 $\mu\mu$M of alanylglycine, an amount that could be hydrolyzed by one sea urchin egg within 20 seconds.

We have improved on Linderstrøm-Lang's[2] method in several ways, for example: density differences as small as 0.000,001 can be measured with a new horizontal measuring microscope; reaction volumes as small as 0.000,5 μL can be safely handled; and, with the volumetric submicromanipulator, samples of cytoplasm ranging from 0.3 to 10 $\mu\mu$L can be extracted from living cells.

Instrumentation

The Dilatometer. This apparatus consists of a stainless steel, plate glass water bath mounted on a platform above a large base which serves as a common support for all components of the dilatometer excepting the circulating pump (FIGURE 1). A 500-watt heater controlled with a Microset differential thermoregulator and an Aminco electronic relay maintains the temperature of the water bath at 30 ± 0.01°C. The water is stirred without vibration by a centrifugal pump mounted independently of the dilatometer assembly and con-

* This paper, illustrated with lantern slides, was the third of three papers in a symposium on Recent Advances in Cytochemistry presented at a meeting of the Section on May 11, 1953.

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‡ These procedures were developed by Dr. Gladys M. Mateyko, Damon Runyon Research Fellow at New York University.

** 1 mL = 10^{-3} liter, 1 μL = 10^{-6} liter, 1 mμL = 10^{-9} liter, 1 $\mu\mu$L = 10^{-12} liter. 1 mM = 10^{-3} mole, 1 μM = 10^{-6} mole, 1 mμM = 10^{-9} mole, 1 $\mu\mu$M = 10^{-12} mole.

†† The horizontal measuring microscope as well as the volumetric submicromanipulator were constructed by Mr. Jack Harris.

nected to inflow and outflow pipes with rubber tubing.

Five glass gradient tubes (capacity *circa* 450 mL) are held in a metal rack which is securely mounted in the water bath. The lower half of the gradient column are thoroughly mixed by vigorous vertical stroking with a wire helix attached to the end of a stiff wire. In about 72 hours, an equilibrium is reached to give a uniform gradient in the column of approximately 0.001/cm. The gradient

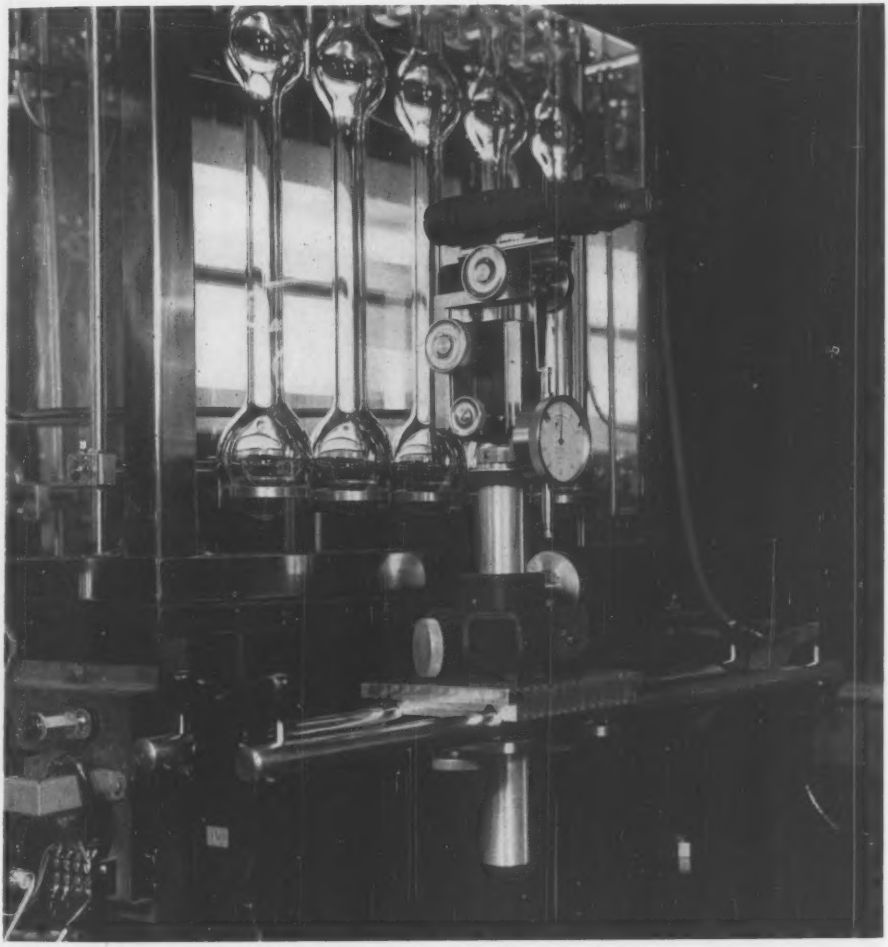

FIGURE 1. The dilatometer.

gradient tube is filled with a mixture of 71 parts refined kerosene and 29 parts bromobenzene, by volume, to give a density of *circa* 1.014. The upper half is then filled with another mixture of 74 parts kerosene and 26 parts bromobenzene to give a density of *circa* 0.994. The fluids in the fluid is equilibrated with water by adding an emulsion consisting of 1 mL of 0.2 M KBr solution dispersed in 10 mL of the lighter gradient mixture. The aqueous drops, due to their high density, sink and eventually rest on the bottom of the gradient tube.

All parts in the bath, including the heater, thermometer, thermoregulator, tube rack, and loading manipulator are mounted on a Flexaframe stand attached to the large base. Behind the bath are mounted two 15-watt fluorescent lamps which illuminate the background for observing the reaction drops.

action drops in the gradient columns. The distances moved by the vertical control are measured directly on a dial micrometer which reads to 0.01 mm over a range of 10 mm.

The microscope with its vertical measuring unit is mounted on a heavy rack and pinion movement so that any part of the gradient column may be

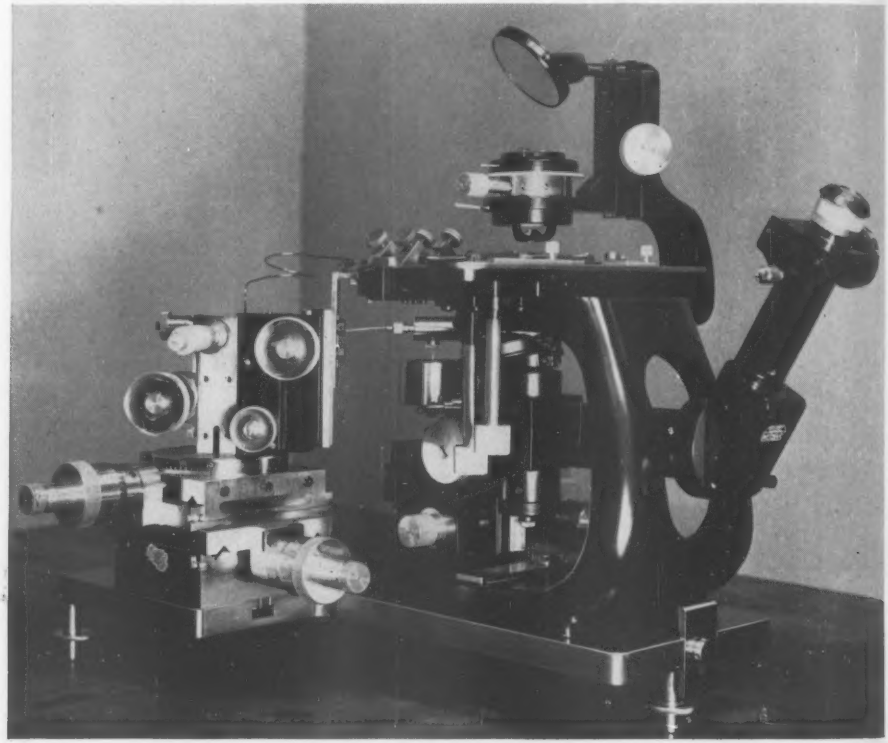

FIGURE 2. Volumetric submicromanipulator.

Horizontal Measuring Microscope. This instrument, designed and built for microdilatometry, is placed in front of the bath (FIGURE 1). The microscope gives an erect image with a magnification of 35× and a working distance of 7.5 cm. A cross hair, placed in the ocular, provides an index for measuring the positions of the reaction drops. The body tube is mounted horizontally on a rack and pinion movement.

This unit is attached to a coarse-fine focusing mechanism for producing the vertical movements needed to follow the changes in position of the re-

surveyed. When the approximate working level is reached, the microscope assembly can be firmly locked in place. The entire unit is clamped to an optical bench so that the microscope can be placed in front of any one of the five gradient tubes.

Placing Reaction Drops into Gradient Tubes. Since it is desirable to have the reaction drops, in a given series, occupy essentially the same cross-sectional area of the gradient column (FIGURE 4), a loader consisting of a Kelly-Richards manipulator was mounted on the gradient tube rack. As shown in FIGURE 3, the enzyme-substrate mixtures

are prepared in a drop of gradient fluid on a cover glass. With the cover glass clamped to the manipulator, the reaction drops may be precisely placed in the desired cross-sectional area with the two horizontal movements. The cover glass is lowered into the gradient tube with the coarse vertical movement until contact with the gradient fluid is established. The cover glass is then oriented by hand so that the two reaction drops are equidistant from the meniscus of the fluid. Then the cover glass is lowered into the fluid with the fine vertical movement. When the reaction drops have moved, by gravity, below the lower edge of the cover glass,

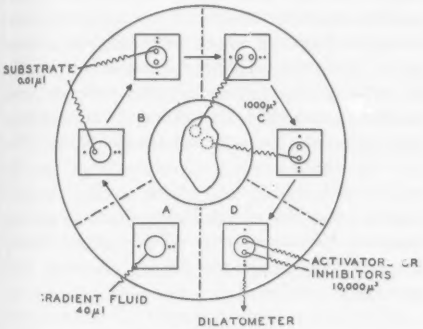

FIGURE 3.

the empty cover glass is withdrawn with the coarse vertical movement. Thus, the reaction drops may be lined up as shown in FIGURE 4.

Volumetric Submicromanipulator. The present model, as developed by Kopac and Harris,[4] has two micromanipulator units integrated with volumetric controls mounted in front of an inverted microscope (FIGURE 2). Each micromanipulator unit has two massive lateral cross movements consisting of spring-loaded, ball-bearing slides to which motion is imparted by metric screws equipped with large micrometer heads. The vertical movement is a modified Bausch and Lomb focusing mechanism on which a bracket is mounted for supporting the micropipette clamp.

The volumetric component is based on the principle of displacing a measured volume of silicone oil in a chamber by one of two pistons which provide either a coarse or fine displacement. The piston chamber is connected to the micropipette holder with flexible copper tubing. This chamber and tube are filled, using the coarse volumetric control, with a viscous silicone oil (GE 9981 LTNV-70). The pipette holder and glass micropipettes are filled with a less viscous silicone oil (DC-200, 3–10 centistokes). The coarse volumetric control consists of a metric micrometer head with its spindle used as a piston operating in a ground and lapped barrel.

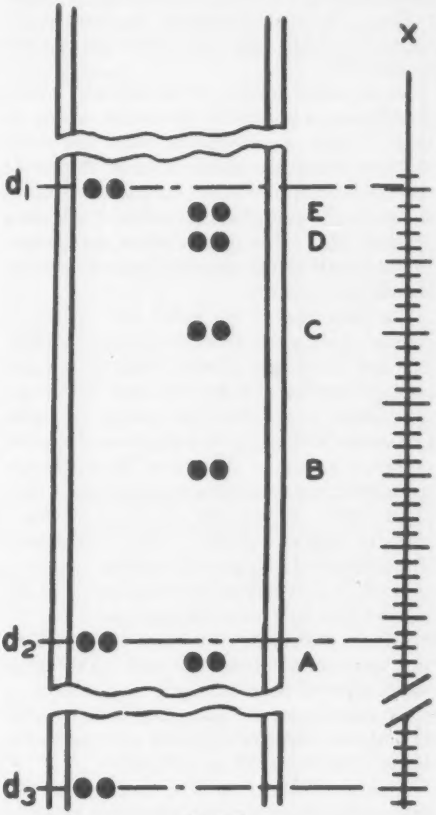

FIGURE 4.

The fine volumetric control on the left side has a high-speed steel piston 0.005 inch in diameter, the one on the right side is 0.0158 inch in diameter. The pistons, obtained from the Van Keuran Company, are class A cylindrical standards made to a tolerance of ±0.000,01 inch. The pistons operate in lapped steel barrels, in which the length of contact between the piston and barrel is six to eight times the diameter of the piston.

The movements of the fine pistons are regulated by mechanisms consisting of a feed screw and worm

gear drive equivalent to a screw with 125,000 threads/inch. One full turn of the volume control knob moves the piston 8 microinches. The linear displacement of the piston is measured with an electronic micrometer which consists of the RCA 5734 Mechano-electronic transducer tube (not shown in FIGURE 2) with one stage of DC amplification. Linear displacements as small as 0.1 microinch (=0.03 $\mu\mu$L with smaller piston) can be measured.

An important feature of the electronic circuit permits one to pre-set the instrument, within its range, to any given volume. When the piston has been moved the correct distance to provide the pre-determined volume, an audible signal is automatically emitted by the action of a Weston Sensitrol relay. This feature allows the operator to concentrate on the removal of cytoplasmic substances from the cells.

The glass pipettes are sealed into the micropipette holders with a Teflon compression coupling. The tips of the glass pipettes, made with a fire-polished opening in a De Fonbrune microforge, are designed to penetrate cells readily. The inside and outside surfaces of the pipettes are siliconized (Desicote) to reduce the sticking of cytoplasmic residues to the glass. The micropipette is completely filled with DC-200 silicone oil having a viscosity ranging between 3 and 5 centistokes. The difficult and laborious task of filling the pipette with oil is conveniently accomplished with the motor-driven drop-retraction apparatus.[5]

A moist chamber of new design accommodates two square cover glasses (22 mm). The top of the chamber can be raised to permit insertion of the cover glasses from the rear position without disturbing the micropipettes which are mounted in front.

A useful feature is that the pipette clamp and volumetric control for that pipette are mounted on opposite micromanipulator units. Thus, the micropipette may be inserted into the cell by manipulating the position controls with one hand and, at the same time, the volumetric control can be manipulated with the other. The movements of the micromanipulator are so precise that there is no difficulty in placing the tip of the micropipette into any selected part of a cell. The new Gamma inverted microscope with Newton long working distance objectives provides magnifications up to 800×.

Limitations of the Dilatometric Method

A difference in density of 0.000,001 can be measured with our present instruments. The size of the reaction volume is also an important factor in establishing the ultimate sensitivity of the method. For example, the alanylglycine peptidase activity of pelomyxan cytoplasm is 4 $\mu\mu$M/hour/$\mu\mu$L. The calculated times required for 1 $\mu\mu$L of this cytoplasm acting on 0.1M L-alanylglycine to increase the density by 0.000,001, at several reaction volumes, are: 0.1 μL, 159 minutes; 0.01 μL, 15.6 minutes; 0.000,5 μL, 0.8 minute.

We use the precision micromanipulator[6] with micrometer controlled injection apparatus for preparing measured volumes of the substrate mixtures in a drop of gradient fluid placed on a cover glass. With this equipment, there is no problem in forming or handling reaction volumes even smaller than 0.000,5 μL. Owing to the relationship between particle size and sedimentation velocity, the smallest reaction volume that can be safely used is 0.01 μL. With smaller reaction volumes, the rate of fall in the gradient column would be limited more by sedimentation velocity than by the changes in density resulting from enzymatic activity.

Procedure

Preparing Reaction Mixtures. Linderstrøm-Lang's method[3] of setting up enzyme-substrate mixtures is too crude to be used for the small cytoplasmic samples that can be obtained with the volumetric submicromanipulator. A new procedure was devised which permits one to add measured amounts of enzyme (as cytoplasmic substance) to substrate volumes as small as 0.000,5 μL. Also important is the fact that the reactions can be run in duplicate.

On FIGURE 3 is a diagram showing the procedures. In A, 40 μL of the lighter gradient fluid (equilibrated with water) is placed in the center of the square cover glass (22 mm). Two marks (.) and (..) are placed on the outside of the gradient fluid as shown in the diagram. In B, the cover glass is transferred to the moist chamber on the stage of the precision micromanipulator. By means of the special microinjection apparatus, two drops each containing exactly 0.01 μL of the buffered substrate mixture are deposited approximately 1.5 mm apart in the gradient fluid. The aqueous mixtures assume spherical shapes, the diameters

of which may be conveniently measured with a filar ocular micrometer.

In C, the cover glass preparation is transferred to the moist chamber mounted on the stage of the volumetric submicromanipulator. Here a measured volume (0.3 to 10 $\mu\mu$L) of cytoplasmic substance is transferred from the cell to the substrate, first to mark (.) and then another to mark (..). The experimental time is designated as zero at the instant the cytoplasmic residue is added to the substrate.

In D, activators or inhibitors, if used at low concentrations are added to the enzyme-substrate mixtures with the larger of the two volumetric submicropipettes. If higher concentrations are required, the activators or inhibitors are incorporated directly with the substrate mixture (step B). From here, the cover glass is transferred to the loading manipulator on the microdilatometer and the reaction mixtures are then inserted into the gradient column.

If a series involving activators and inhibitors is tested, the reaction mixtures are placed into the gradient tube in order of their anticipated activity. The most active preparations (highest density) precede the less active preparations. As shown in FIGURE 4, the fully hydrolyzed substrate, A, is of the same molarity as used in the other reaction mixtures. The others, placed into the gradient fluid at intervals of 15 minutes are the following: B. substrate + enzyme + activator; C. substrate + enzyme; D. substrate + enzyme + inhibitor; and E. substrate.

The expected relative density changes of the four reaction mixtures and of the hydrolyzed substrate are shown in FIGURE 5. The spontaneous hydrolysis of the substrate is indicated by E.

Measuring Density Changes. As noted in FIGURE 4, the gradient columns are calibrated with a series of known densities, d_1, d_2, and d_3, for example. The calibration drops with a volume of 0.01 μL are made in duplicate from various concentrations of KCl using the same steps as described for preparing the substrate mixtures. These drops are also placed into the gradient column with the loading manipulator.

The first measurement is made 30 minutes after placing the pair of reaction drops into the gradient column. By this time, it is assumed that the drops have reached a level in the gradient column equal to their density. Before the first measurement is made, the cross hair in the horizontal microscope is lined up with the nearest calibration drops above the reaction drops, for example, on d_1 in FIGURE 4. The dial micrometer is then set to zero. The distance, x, between the reaction drops and d_1 is measured at intervals of 30 minutes. If more than one set of drops is used, it is customary to place them into the gradient fluid at intervals of 15 minutes. These are also measured at intervals of 30 minutes.

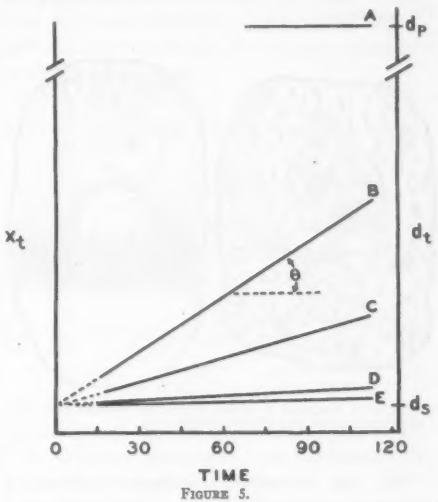

FIGURE 5.

The x-values, in units of 0.01 mm, are plotted against time, t, as shown in FIGURE 5.

Calculations. The following equations are required to calculate the activities: (1) $\Delta M/\Delta t = (\Delta d/\Delta t)\ v/kd_s$; (2) $\Delta d/\Delta x = (d_2 - d_1)/x$; (3) $d_t = d_1 + (\Delta d/\Delta x)\ x_t$; (4) $\Delta d/\Delta t = (\Delta d/\Delta x) \tan\theta$; (5) $k = [(d_p/d_s) - 1]\ v/\Delta M$.

In the above, ΔM = micromoles (μM) of substrate hydrolyzed; Δd = change in density; Δt = time interval, usually 1 hour; v = volume of reaction drop in mμL; $\Delta d/\Delta x$ = density gradient; d_1 and d_2 = calibration densities; x = distance between d_1 and d_2 in cm; d_t = density of reaction drop at any time, t; x_t = distance between d_1 and position of reaction drop at any time, t; $\tan\theta = \Delta x/\Delta t$; k = constriction constant in mμL/μM; d_p = density of completely hydrolyzed substrate; and d_s = density of substrate at $t = 0$ (obtained by extrapolating the density-time curve to $t = 0$).

Localization of an Enzyme in the Cell

The following general account illustrates the principles involved in localizing an enzyme in the cell using the methods outlined herein. On FIGURE 6 are diagrams of optical sections of isolated liver cells (*Amphiuma*), before and after centrifugation at 20,000 G for 90 minutes. The cytoplasmic particulates have become rearranged to form the following layers: I (top), lipids; II. matrix; III. mitochondria, nucleus, secretory granules, matrix; IV. glycogen.

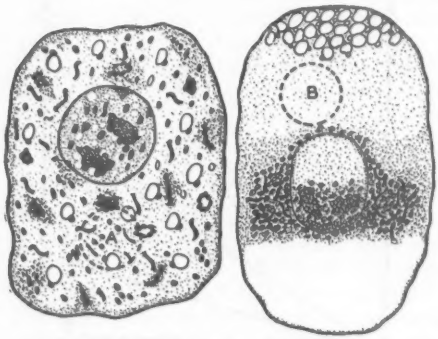

FIGURE 6. Diagrams of optical sections of isolated liver cells (*Amphiuma*) before and after centrifugation.

With the volumetric submicromanipulator, a measured volume of cytoplasm can be removed from the uncentrifuged cell and transferred to a substrate mixture. This sample, A, contains various particulates (with exception of nucleus) in accordance with their distribution in the cell. From the centrifuged cell, exactly the same volume of matrix, B, may be similarly removed and transferred to the substrate mixture. The activities of the enzymes from these sources are then measured in the dilatometer.

For example, the activity of sample A with the substrate used may be shown by line C in FIGURE 5. Although this graph shows the changes in density with time, from which the activity in μM/hour can be calculated, the activity, nevertheless, is proportional to the slope.

Suppose the activity of the matrix fraction, B, were indicated by line B. This would indicate that the enzyme is localized in the matrix fraction. Association of the enzyme with the larger particulates would have produced an activity similar to line D or possibly E.

If the activity were exclusively associated with the matrix, it would then be possible to calculate the specific activity of that fraction.[7] Knowing the specific activity, one could then calculate how much matrix was actually present in sample A and, accordingly, one would also know how much particulate material was present in that sample.

Methods for determining the enzymatic activities of the nucleus as well as of other subcellular particulates have been devised.[7, 8, 9] The key to the localization of enzymes in the cell is to find out what enzymes are specifically present in the matrix. By centrifugation, one may get matrix fractions uncontaminated by other particulates. It is impossible to get particulate fractions that are uncontaminated by matrix.

It should be emphasized that the separation of various particulates from one another in the cell may be accomplished without any irreversible injury either to the particulates or to the cell. In fact, the cytoplasmic sample is alive until the very instant physical separation of the sample from the bulk of the cell is accomplished.

Enzymatic activities that may be measured by these procedures include all proteinases, peptidases, phosphatases, pyrophosphatases, and nucleic acid depolymerases. Similarly, measured volumes of cytoplasm may be used as substrates for ATP-ase, with or without myokinase, nucleic acid depolymerases, with or without proteinases, and for other enzymes. The same procedures, in fact, can be used for measuring synthetic reactions, for example: synthesis of peptide bonds, nucleotide polymerization, phosphorylation, *etc.* In these reactions, there would be an increase in volume and, accordingly, a decrease in density.

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SECTION OF MATHEMATICS AND ENGINEERING

ON THE GENERAL SOLUTION OF A WAVE EQUATION*

By W. S. JARDETZKY†

The solutions of wave equations were presented in many different forms and the aim of this paper is to discuss the question of the general solution. According to the classical definition given by Darboux a solution of a partial differential equation is a general integral if it is expressed in terms of arbitrary functions or of an infinite number of arbitrary constants in such a way that one can derive from it all solutions the existence of which is proved by Cauchy theorem.

A wave equation

$$\nabla^2\phi = \frac{1}{\alpha^2}\frac{\partial^2\phi}{\partial t^2} \tag{1}$$

belongs to the class of homogeneous linear partial differential equations of the second order with constant coefficients, if α = const., *i.e.*, if the medium is homogeneous. To solve equations of this class Cauchy made use (1821) of Fourier integrals and gave (1831) the solution of the wave equation in the form

$$\phi = \frac{1}{(2\pi)^3}\int_{-\infty}^{\infty}\overset{(6)}{\cdots}\int\left[\bar{\omega}(\hat{x},\hat{y},\hat{z})\cos\theta t + \Pi(\hat{x},\hat{y},\hat{z})\frac{\sin\theta t}{\theta}\right] x \exp\{i[v_1(\hat{x}-x) + v_2(\hat{y}-y) + v_3(\hat{z}-z)]\} \, dv_1\, dv_2\, dv_3\, d\hat{x}\, d\hat{y}\, d\hat{z} \tag{2}$$

where θ is determined by the algebraic equation $g(v_1, v_2, v_3, \theta) \equiv v_1^2 + v_2^2 + v_3^2 - \frac{\theta^2}{\alpha^2} = 0$. There are two arbitrary functions of parameters v_i, $\hat{x}$, $\hat{y}$, $\hat{z}$ in the expression (2) and these parameters are eliminated from the final expression by integration.

The Poisson solution of the Cauchy problem and the Kirchhoff formula yielded new forms of solutions of a wave equation. According to Whittaker[17] the general solution of a wave equation can be written in the form

$$\phi = \int_{-\pi}^{\pi}\int_{-\pi}^{\pi} f(x\sin u\cos v + y\sin u\sin v + z\cos u + \alpha t, u, v)\, du\, dv \tag{3}$$

where f is an arbitrary function and u, v two parameters. It was shown by Bateman[1] that the expression

$$\phi = \int_{-\pi}^{\pi} F(x\cos\theta + y\sin\theta + iz, y + iz\sin\theta + \alpha t\cos\theta, \theta)\, d\theta \tag{4}$$

satisfies also (1) if F is an arbitrary function and θ an arbitrary parameter. The expression (4) is also called a general solution.

Some conditions of a problem may require a larger number of parameters than those used in (3) or (4) and Jardetzky[7] suggested another form of a general solution of a wave equation. This form is an extension of (3) and is given by the following equation:

$$\phi = \sum\int_{(L)}^{(7)}\cdots\int f_i(\psi_i; v_1, v_2, v_4, \hat{x}, \hat{y}, \hat{z}, \hat{t},) \, dv_1\, dv_2\, dv_4\, d\hat{x}\, d\hat{y}\, d\hat{z}\, d\hat{t} \tag{5}$$

where f_i are two arbitrary functions of the arguments

$$\psi_{1,2} = v_1(x-\hat{x}) + v_2(y-\hat{y}) \pm v_3(z-\hat{z}) + v_4(t-\hat{t}) \tag{6}$$

$$\text{provided} \quad v_3 = \sqrt{\frac{v_4^2}{\alpha^2} - v_1^2 - v_2^2}. \tag{7}$$

The limits of integrals in (5) can be equal to $\pm\infty$, but some other values are not excluded

* This paper, illustrated with lantern slides, was presented at a meeting of the Section on May 15, 1953.

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a priori. A simple summation with respect to any parameter instead of the integration can obviously take place in a particular case. It was shown (Jardetzky[7]) that several other forms of integrals[4, 5, 9, 11] used to solve a wave equation can be derived from (5).

We will now discuss some other forms of solutions of a wave equation. It should be noted that the arbitrary elements involved in the solution (5) are; two functions, seven parameters, and the limits of integration.

The possibility of a generalization of (4) is evident. We can put, namely,

$$\phi' = \int \overset{(7)}{\cdots} \int F(\psi', \psi''; v_2, v_4, v_6, \hat{x}, \hat{y}, \hat{z}, \hat{l})\, dv_2\, dv_4\, dv_6\, d\hat{x}\, d\hat{y}\, d\hat{z}\, d\hat{l} \quad (8)$$

where

$$\psi' = v_1(x - \hat{x}) + v_2(y - \hat{y}) + v_3(z - \hat{z}),$$

$$\psi'' = v_4(y - \hat{y}) + v_5(z - \hat{z}) + v_6(t - \hat{l}). \quad (9)$$

The function F will be a solution of the wave equation (1) if three conditions are satisfied as follows

$$v_1^2 + v_2^2 + v_3^2 = 0, \quad v_2 v_4 + v_3 v_5 = 0,$$

$$v_4^2 + v_5^2 = \frac{v_6^2}{\alpha^2}. \quad (10)$$

This is directly verified by inserting F in (1). There are thus seven independent parameters of ten: v_i, $\hat{x}$, $\hat{y}$, $\hat{z}$, $\hat{l}$ and the summation of F with respect to these variables represents the required generalization. It is obvious that the expression (8) is one of the terms in the solution which we will obtain if we express v_1, v_3, v_5 in terms of other parameters. Bateman[3] made use of expressions similar to (9 & 10) where only the parameters v_i are involved. In order to avoid any confusion we have to note that there are, in all forms of general solutions, one or more "arbitrary functions" used. This term, however, does not involve an integration with respect to some parameters which eliminates these parameters from the final expression of the function.

The use of the exponential function instead of an arbitrary one in the expression (5) is well known and if we do not yet specify the sign of v_3 in (6) we can write one term in (5) in the form

$$\phi = \int \overset{(7)}{\cdots} \int F(v_1, v_2, v_4, \hat{x}, \hat{y}, \hat{z}, \hat{l})\, e^{i\psi}\, dv_1\, dv_2\, dv_4\, d\hat{x}\, d\hat{y}\, d\hat{z}\, d\hat{l}. \quad (11)$$

In general, there are no restrictions concerning the nature of parameters v_i. They can be real or complex. In the latter case we can obtain attenuated waves for the corresponding variable. Now, if we assume that there is one harmonic source acting at the origin it is easy to see the changes in notations in (11) which are necessary to obtain the Weyl's[16] integral

$$\frac{1}{R} e^{i(\omega t - \kappa R)} = -\frac{i\kappa}{2\pi} e^{i\omega t} \int e^{-i\kappa(\alpha x + \beta y + \gamma z)} d\Omega. \quad (12)$$

It is in this integral k a real and positive constant, $\alpha = \sin\theta \cos\phi$, $\beta = \sin\theta \sin\phi$, $\gamma = \cos\theta$, $d\Omega = \sin\theta\, d\theta\, d\phi$ and the integration is over the upper half of the imaginary unit sphere: $\phi(\theta, 2\pi)$, θ changing from 0 over $\frac{\pi}{2}$ to $\frac{\pi}{2} + i\infty$ in the complex θ plane; $R^2 = x^2 + y^2 + z^2$. The more general solution

$$\phi = \frac{1}{R} f\left(t - \frac{R}{\alpha}\right) \quad (13)$$

was written by Poritsky[12] in the form of an extended Weyl's integral. Since this generalization is obtained by Fourier transformation, it can be also derived from the expression (5). Similar conclusions hold obviously for the solutions of the type

$$\phi = \frac{1}{R} f_1\left(t + \frac{R}{\alpha}\right) \quad (14)$$

as well as for some of those derived from the fundamental (or elementary in the sense given by Hadamard) solution $1/R$ of Laplace equation like

$$\phi = \frac{1}{2R}\left(\frac{1}{R - \alpha t} + \frac{1}{R + \alpha t}\right) = \frac{1}{R^2 - \alpha^2 t^2}. \quad (15)$$

On making use of cylindrical coordinates r, z, and, therefore, of the Bessel function J_0 the solution of

the type (12) was written by Lamb[9] and Sommerfeld[14] in the form

$$\frac{1}{R} e^{i\omega/\alpha R} = \int_0^\infty J_0(\lambda r) e^{-v|z|} \frac{\lambda \, d\lambda}{v} \quad (16)$$

where $v^2 = \lambda^2 - \frac{\omega^2}{\alpha^2}$ and v must have a positive real part in order to insure the convergence of the integral and its vanishing at $z \to \pm \infty$; the time factor is omitted in (16). The Lamb-Sommerfeld expression can be derived from that of Weyl by integration of the latter with respect to the angle ϕ.

The expression (16) corresponds to a spherical wave from one point source and a more general solution concerning the propagation of electromagnetic or elastic waves in layered media taken in the form

$$\phi = e^{-i\omega t} \int_0^\infty F(\lambda) J_0(\lambda r) e^{-v|z|} \, d\lambda \quad (17)$$

is obviously also a particular case of (11). Some modified forms of (16) or (17) were used in particular problems as well as such with surface integrals (Niessen[11]), but they are not more general, for example, than the expression (17).

A special attention must be paid to some other solutions considered by Bateman,[2, 3] Volterra,[15] Smirnov and Sobolev,[13] and others.

Whittaker[17] gave to the general solution of Laplace equation a form similar to (3) and a formula of the type (5) can also be written for this equation. The number of parameters will be obviously smaller than that in (5). Jacobi[3] seems to be the first to show that if in an expression like (6) we will call ψ a new parameter and assume that v_i are functions of it, this variable becomes a function of x, y, z determined by the equation

$$\psi = xv_1(\psi) + yv_2(\psi) + zv_3(\psi). \quad (18)$$

An arbitrary function $F(\psi)$ is then a solution of Laplace equation provided $\Sigma v_i^2 = 0$. A similar assumption was applied by Volterra to the wave equation in a two-dimensional case and later in the method of Smirnov and Sobolev in two- and three-dimensional problems. On making use of expressions

$$\theta \equiv \psi = \sum_1^m x_i v_i(\theta) \quad \text{or} \quad \psi(\theta) = xv_1(\theta) + yv_2(\theta) + zv_3(\theta) \quad (19)$$

Bateman called primary solutions those of the type $F(\theta)$ and noted that they can be generalized by integrating with respect to a parameter after they are multiplied by an arbitrary function of it. However, the number and role of such parameters were not indicated.

If we assume that a function θ is determined by the equation

$$\psi(\theta) = xv_1(\theta) + yv_2(\theta) + zv_3(\theta) + tv_4(\theta) \quad (20)$$

which we write instead of (6) and that the condition (7) holds also for (20), a new interpretation must be given to the results. In (5 & 6) the condition ψ = const. is leading to the idea of "superposition" of plane waves, since if $v_i, \dot{x} \cdots$ are constant this condition represents a set of planes. Now, if we take a root of (20) θ^* which is a certain function of x, y, z, t and put

$$\theta^*(x, y, z, t) = \text{const.} \quad (21)$$

the corresponding surface or a set of surfaces determined by the parameter t will in general differ from planes. Moreover, there will be several families of surfaces, since the equation (20) can have different roots. Thus, on using (20) one will avoid the interpretation of the general solution as due to superposition of plane waves and, of course, has to reduce the number of integrations in (5) because of the fact that v_i are now functions of a single parameter θ. The roots (21) can be real or complex and it is quite natural to consider the plane of a complex variable θ. In two-dimensional problems, for example, (20) takes the form

$$t - \theta x \pm z \sqrt{\frac{1}{\alpha^2} - \theta^2} = \psi(\theta). \quad (22)$$

It is usually assumed that $\psi(\theta)$ is an analytical function and it was proved by Smirnoff and Sobolev that if θ^* is real any function of it having differential coefficients of first two orders satisfies the wave equation and that the real part of an analytical function $F(\theta)$ is the solution of a wave equation if $\theta = \theta^*$ is complex. In both cases the equation of characteristics is satisfied too. Each root θ^* corresponds to a wave front and it seems to be possible to rewrite the equation (5) in the form

$$\phi = \sum Re \int f_i(\theta_i^*; \hat{x}, \hat{y}, \hat{z}, \hat{t}) \, d\hat{x} \, d\hat{y} \, d\hat{z} \, d\hat{t}. \quad (5')$$

Then, one part of the sum in the rightside member will correspond to a discrete spectrum of roots of (20) but the second part due to the continuous spectrum can also exist.

The use of a single linear function of coordinates and time like (20) is not a necessary condition for solution of a wave equation. This fact is expressed in the Bateman's solution (4), and by introducing new appropriate conditions other expressions can be written which will satisfy a wave equation. For example, if we put instead of (9)

$$\psi_i = v_{1i}x + v_{2i}y + v_{3i}z + v_{4i}\alpha t + v_{5i}, \qquad i = 1, 2, \cdots n \tag{23}$$

the function $$\phi = \frac{1}{\psi_1 \psi_2 \cdots \psi_n} \tag{24}$$

will satisfy the wave equation (1), provided

$$v_{1i}v_{1j} + v_{2i}v_{2j} + v_{3i}v_{3j} = v_{4i}v_{4j}; \qquad i, j = 1, 2, \cdots n \tag{25}$$

On assuming that v_{ij} are functions of two parameters σ and τ Bateman[3] considered the solution

$$\phi = -\frac{1}{4\pi^2} \iint \frac{f(\sigma, \tau)\, d\sigma d\tau}{\psi_1 \psi_2} \tag{26}$$

represented by a double contour integral. The use of more than two factors in (24) yields a simple generalization of (26).

Another type of solution is written by Bateman in the form

$$\phi = \zeta f(\xi, \eta) \tag{27}$$

where the functions ξ, η and ξ have to satisfy certain conditions which one can easily see on substituting (27) into wave equation. One of these conditions is the equation of characteristics

$$(\text{grad } \phi)^2 = \frac{1}{\alpha^2}\left(\frac{\partial \phi}{\partial t}\right)^2 \tag{28}$$

It is obvious that introducing a certain number of complementary conditions we will be able to write new expressions which will satisfy these conditions and the wave equation. This does not always increase the generality of such an integral of the wave equation. From the physical viewpoint, among these conditions, the most essential is the characteristic equation (28) because it must be satisfied on the wave fronts, *i.e.* on the surfaces of equal phase or surfaces of discontinuity of some kind. In cases of a solution of Whittaker's type or (5) the physical interpretation is immediate because of the use of wave fronts. Less evident is the interpretation of the Bateman's solution because of increased number of conditions (10). Nevertheless, under these conditions the solution (4) satisfies also the characteristic equation. As to the wave fronts, it was pointed out by Love[4] that there are three types of waves with boundaries; (1) when neither ϕ nor its differential coefficients of the first order are discontinuous at the boundary, (2) the function ϕ is continuous, the differential coefficients are discontinuous, and (3) the function ϕ is discontinuous. If this boundary is advancing with a velocity α and n is the direction of the normal, the condition to be satisfied is, as is well known,

$$\frac{\partial \phi}{\partial t} \pm \alpha \frac{\partial \phi}{\partial n} = 0 \tag{29}$$

Thus, the equation (28) is equivalent to two equations (29).

The very important question of the role of limits in integrals was still left out of consideration. If we make use of Fourier integrals the limits are $\pm\infty$, but in such cases as equations (5) or (8) one cannot see *a priori* the necessity to use, always, the same definite limits. Bateman[2] made attempts to introduce some other limits than infinity or $\pm\pi$. He discussed the integral

$$\phi = \int_{\theta_1}^{\theta_2} f(x, y, z, t, \theta)\, d\theta. \tag{30}$$

If the function $f(x, y, z, t, \theta)$ satisfies the wave equation for all values of the parameter θ this integral will be a solution. The problem was posed to find a suitable pair of functions $\theta_1(x, y, z, t)$ and $\theta_2(x, y, z, t)$ such that the integral (30) will represent a solution of a wave equation. On substituting the expression

$$\phi = \int_{\theta_1}^{\theta_2} \zeta F(\xi, \eta, \theta)\, d\theta \tag{31}$$

where ξ, η, ζ depend on x, y, z, t, into wave equation, Bateman obtained a system of partial differential equations for θ_1 and θ_2 which are difficult to solve. The conditions for the use of variable limits are, however, important in applications and deserve a special attention.

Even if we will not make use of variable limits in

(5) this expression seems to possess the generality required in physical problems. There are two arbitrary functions involved, the wave equation being of the second order. The number 7 of arbitrary constants is equal to that in a complete integral for these type of equations. Moreover, if we want to represent any function which is a solution of the wave equation (1) by Fourier integrals, the number of independent constants will be the same, one more than in Cauchy's expression (2). These indirect considerations do not show, of course, whether the general solution taken in the form (5) corresponds to the definition of Darboux or not. On making use of an expression of the type (5) for two independent variables and one parameter Goursat[6] pointed out that if f does not satisfy some special conditions this expression will not necessarily be an integral in the sense of Darboux' definition.

The representation of a general solution by an equation of the form (5) obviously does not depend on the number of variables and can easily be generalized for any homogeneous partial differential equation with constant coefficients, as was recently shown by the author.[8]

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*SECTION OF PSYCHOLOGY**

IMPLICATIONS OF RECENT STUDIES ON INTELLIGENCE†

By ROBERT G. BERNREUTER‡

The differences in the viewpoints which psychologists hold toward intelligence are as broad as the Atlantic Ocean. It seems that the English and the Americans simply cannot agree on the nature of intelligence.

A controversy has been going on between them for a half century, in fact ever since Charles Spearman devised his famous two factor theory which accounted both for the fact that correlations among test scores were positive, and the further fact that while they were positive they were not unity.

This theory was in sharp contrast with the American findings published just three years earlier. Wissler had given a battery of Cattell's tests to college students at Columbia University. He reported that the tests had such low intercorrelations and such low correlations with college marks that each seemed to be measuring something quite unique, with nothing in common. Hence, he doubted the existence of a "general" intelligence and advocated the theory that psychological tests merely measured a variety of independent special abilities.

These American findings partially supported the views of Binet in France who advocated the theory of separate faculties, such as memory, imagination, attention, perception, and reasoning. Binet's personal concept of "general" intelligence is much misunderstood today. He conceived of "general" intelligence as an average of several separate faculties. On his intelligence scales the mental age was simply the average level attained on a series of quite separate and independent functions. Binet was unwilling to accept Spearman's theory

* The Division of Mycology held a meeting on May 22, 1953 at which a paper entitled "A Microscopic Study of Experimental Fungus Infections in Humans due to *Microsporum canis* and *M. audouini*," was read by Albert M. Kligman. An abstract of this paper will be published in a later issue.

† This paper was presented at a meeting of the Section on May 18, 1953.

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that all mental tests require one common factor. He was unimpressed by Spearman's work and almost wholly uninfluenced by it.

The positions assumed by the British, the French, and the Americans fifty years ago are still maintained by the principal investigators in these countries. As examples, I might cite the results of the factoral studies in the British Military Service during the last war. They found that more than two-thirds of the commonality among their classification tests was due to "g." On the other hand, the recent studies of Meili, published in French, report no general factor, but do report a number of broad so-called multiple factors operating within a battery of ability tests. Also, in a review of the American work, John French lists 59 group factors, none of which is broad enough to be considered a general factor.

Three viewpoints, which represent attempts at compromise between these extreme views, are currently advocated and are important enough for us to consider here. These are the hierarchy viewpoint, the emergent factors viewpoint, and the second-order factors viewpoint. In addition, a fourth viewpoint is important even though it does not represent a compromise. It is the orthogonal simple-structure concept, which modifies Binet's view but does not move toward a general factor.

The hierarchy viewpoint goes back to Herbert Spencer, in England, and has recently been advocated by his fellow Englishmen, Alexander, Burt, and Vernon. It places a general factor at the highest level, group factors of varying degrees of breadth at the intermediate levels, and individual sensory or motor activities at the lowest and most specific levels.

The emergent factors viewpoint is largely the brain child of Henry Garrett with the Thurstone's filling the role of godparents. Thus it has good American heritage. It holds that early in the developmental history of a child his abilities are undifferentiated and the general factor is of great importance. However, as the child gets older, more specific factors emerge so that by the time he is an adult his mental activity is analyzable into specific factors, and the general factor is of little importance.

The second-order factors viewpoint seems principally to be the invention of Thurstone. This assumes that the various factors, of which intelligence is composed, are not necessarily independent, that they may be related to each other, and hence correlated. Such correlated factors are usually referred to as "oblique" factors. Because they are correlated, their interrelations can be investigated by factor analysis techniques with the certainty that more fundamental factors, the so-called second order factors will be disclosed.

The popularity of the use of oblique factors is shown by John French's review. He found that in 27 out of the 69 studies, oblique factors were obtained.

The orthogonal simple structure viewpoint is also Thurstone's contribution. It conceives of a number of broad independent factors which may function *simultaneously*, in various combinations, as a person goes about his tasks. Like Binet's view, it assumes a number of independent factors, but unlike Binet's view, which assumed that each act required the functioning of only one faculty, it assumes that each act may require the simultaneous functioning of several factors.

There is evidence for and against each of these viewpoints. In the latest issue of the British Journal of Psychology, Statistical Section, there appears an article by E. M. Moursy entitled "The Hierarchical Organization of Cognitive Levels." He gives four sensori-motor tests, five perceptual tests, four associative tests, and seven relational tests to 166 boys, ten years of age, in London schools.

He computed the intercorrelations among the test scores. These he analyzed by nine different factor analysis techniques in order to determine which technique would best account for the intercorrelations. He found that Cyril Burt's subdivided bipolar technique gave the best fit. From this, he concluded that the most appropriate hypothesis envolved a hierarchical arrangement. At the apex of the hierarchy, he placed General Cognitive Ability. On the next lower level, he placed two broad factors which he named Practical and Intellectual Abilities. On the third level, he placed the four types of processes with which he started, the sensori-motor, the perceptual, the associative and the relational processes.

As an illustration of the success of the analysis, let us consider the Otis test, which was found to have the highest saturation of the general cognitive ability. By totaling the contributions of all of the factors, we find that 87 per cent of the variance of the Otis test scores was accounted for. This was made up of a large loading of general cognitive ability and fairly large loadings of the intellectual and the relational factors. The practical, the

sensori-motor, the perceptual and the association factors made only minor contributions. All this is quite understandable to anyone who is familiar with the Otis and lends credence to the whole hierarchy concept.

One of the nine methods of analysis which he used was Thurstone's method of rotation to simple structure. This is the favorite technique here in America and is used in the great majority of American factoral studies. Moursy's simple structure consisted of five factors. One of these he was somewhat inclined to call a general factor because all of the loadings were positive but it seems hardly fair to interpret it as such, especially since it was found to account for only one one-thousandth of the variance of the Otis test!

He concluded that rotation to simple structure does not support the hierarchy concept, but also it does not disprove it.

Although Moursy does not report it, his data are sufficiently complete so that it took only a few moments to compute the per cent of the variance on the Otis test which can be accounted for by the factors in the simple structure. This I computed to be 84 per cent, which is only 3 per cent lower than was accounted for by the hierarchy structure. Furthermore, when, for all 20 of the tests used in the study, the average per cent of the variance which was accounted for by the hierarchy was computed, it was found to be 56.7 per cent. For the simple structure, it was found to be 56.1 per cent. Even this extremely slight advantage for the hierarchy was obtained only by including nine factors in the structure whereas there were only five factors in the simple structure. If the comparison is limited to the five factors in the simple structure and to those seven in the hierarchy which made significant contributions, the simple structure accounts for the larger percentage of the variance.

When it is necessary to decide arguments in this field by reference to the third decimal place, it is time to call it a draw. It is true that the hierarchy looks prettier when laid out two-dimensionally on a sheet of paper. But if you are willing to exercise your imagination and wander through five-dimensional space, you will find that the simple structure solution has great beauty too. My personal feeling is that the Moursy study shows clearly that the hierarchy viewpoint is sound and clean, but no more functional than is the discrete factor viewpoint.

Now let us consider the emergent factors viewpoint. We have already said that this is a contribution of Henry Garrett. In his own words Garrett's hypothesis runs as follows, "Abstract or symbol intelligence changes in its organization as age increases from a fairly unified and general ability to a loosely organized group of abilities or factors."

In support of this hypothesis Garrett presented data from a wide variety of studies which showed that the intercorrelations among *tests* given to young children were higher than for similar tests given to older children and to adults. He also presented evidence, largely from Thurstone's early works on factor analysis, which showed that the intercorrelations among *factors* were lower for older children than for younger.

The emergent factors viewpoint has gained adherents. The Thurstones have utilized it to explain the intercorrelations among the battery of primary mental ability tests, which they prepared and which are published by Science Research Associates. They reported a median correlation of .22 between the factors on the 11 to 17 year tests, and of .32 on the 7 to 11 year tests. They do not report data for the youngest level, five to seven years, but Douglas Dean of the Pennsylvania State College does. He reports a median correlation of .53 when the tests were given to a group of 145 first grade children. This decrease of correlation among the factors (.53, .32, .22) as children get older would have been predicted by the Garrett hypothesis.

At Penn State we have been interested in this hypothesis and have devoted quite a bit of study to it. None of the results have been published so it might be interesting for me to report them to you now.

Douglas Dean made a factor analysis of the Stanford Binet and the SRA Primary Mental Abilities Battery at the first grade level. He used 14 Binet subtests from Form L on the six, seven, and eight year levels, and the five factor scores from the Thurstone. He extracted six factors. On five of his six factors, the tests with the highest loadings were from the Binet rather than from the Thurstone. This was surprising, since the Thurstone was built on the basis of a factor analysis, whereas the Binet was not. However, the Binet tests were given as individual tests whereas the Thurstones were given as group tests. It may be that, in attempting to adapt tasks to fit the requirements of group tests, the constructors introduced some variable which serves a general

factor. There appears to be a sort of "group-test taking ability" which affects the group test scores and increases intercorrelations among them but which does not influence scores on individual tests. In general, Dean's conclusions seem to lend the Garrett hypothesis little support.

Another unpublished research on this topic was done at Penn State by Ila Holsopple. She made two factoral studies, one on the fourth grade and another on the eighth grade level. In each she used the Kuhlman-Anderson Intelligence Tests and the Stanford Achievement Tests. On each level, she was able to name five clearly defined factors. On the higher level she extracted two additional factors, as would have been predicted by the Garrett hypothesis. She was unable, however, to name either of the two additional factors. Also an inspection of the residuals indicates that she left more in the fourth grade table than in the eighth; that is, the extra factors she found on the higher level appear to be more apparent than real. She concluded "The existence of separate primary factors is as well-defined for the younger age group as for the older age group."

Another bit of unpublished research on this topic was done by myself, using data reported by McNemar for the revised Stanford-Binet. He reported several factor analyses at various age levels in his volume entitled "The Revision of the Stanford-Binet Scale." He did not rotate his factors, and so was able to conclude that the Stanford-Binet measures a general factor at all levels. After having rotated several of his structures, I was impressed by the fact that in each study, structures were obtained which did not contain general factors. Furthermore, the structures for the younger children were as clear cut and precise as were those for the older children. Since then L. V. Jones has published in Psychometrika further rotations of the same data with similar findings.

None of the studies give the emergent factors theory much support. It is still a respectable theory but it seems to gain strength very slowly. Unless really conclusive evidence is forthcoming soon, it is likely that it will have to be written off as merely another interesting attempt to bring harmony between the general factor and the no-general factor theorists.

The third attempt at compromise is the oblique factor, second-order general factor viewpoint This first came to my attention following our having given Thurstone's experimental form of his factored tests to freshmen at Penn State in 1938. We tested several hundred freshmen, scored their tests, intercorrelated the results, and found to our surprise that the scores on these factored tests correlated positively. True, they were not high correlations, but they were significant. One of our graduate students, Charles Goodman, ran a factor analysis of these intercorrelations among the supposedly independent tests and found that they measured one factor in common! Not only were the tests not independent, as Thurstone's theory would have them be, but worse still, they were measuring a general factor, just as Spearman kept saying they would. It seemed to us then that if the tests that Thurstone built to demonstrate the value of the multiple factor viewpoint were really measuring "g", surely the British had not only won a battle but they had actually won the war!

Soon after, Thurstone himself, published the same finding. Since then he has gone even farther on the road toward compromise by giving up his early insistence on so-called orthogonal factors and by expanding his theory to include oblique factors. This simply means that he believes that primary factors may be correlated and still be useful in explaining the correlation found among tests. The extremities of his compromise is shown in this sentence from his book "Multiple Factor Analysis." "It seems likely that a second-order general factor, determined from correlated primaries, may turn out to be Spearman's general intellective factor 'g'."

Despite this admission that "g" may have an existence behind the primary mental abilities found by multiple factor analysis, the Thurstone group still tends to ignore the general factor as much as possible. You will recall that, a little earlier, I said that in French's review he reported that 27 recent factoral studies found oblique factors. Only one of these 27 studies was carried through to the computation of second order factors, although presumably each of the other 26 could have been. Interestingly enough, in this one instance the second-order results turned out to be not one general factor, but four broad group factors. Many more studies must be carried through to a complete analysis before we can be sure of the ultimate nature of second or higher order factors.

Anyone who wishes to make practical contributions to intelligence test construction today must first decide what his position is to be regarding the

various conflicting theoretical viewpoints. In our own test construction work at Penn State, we have adopted the orthogonal simple structure viewpoint, without any of the three compromises that we have been mentioning. What we are attempting to do is to select tests which are at least relatively pure factorially, and which measure the most significant factors.

Currently, we are devising tests for the memory, verbal and fractionization factors. The work of Harold Corter, who recently authored a Psychological Monograph entitled "Factor Analysis of Some Reasoning Tests," has helped us. Some of the results of his work and of my own re-rotation of his data were reported at the Eastern Psychological Association Meeting in Boston last month. The work on Factor F-Fractionization is an outgrowth of this study.

Corter gave 21 individually administered tests, principally of reasoning, to 100 high school juniors, and out of the table of intercorrelations he extracted eight factors. He rotated these to simple structure, and later I rotated them to an alternate simple structure. Of the eight factors, four reasoning factors, I-Induction, D-Deduction, Sy-Synthesis and F-Fractionization were found by the re-rotation.

One of these has been given very little attention in the past. At least, no commonly accepted name has been given to it. The essence of the factor is the breaking down of a gestalt in order to extract a sub-gestalt. Because the process involves the fractionizing of a visual pattern, the name Factor F-Fractionization was suggested by us for it.

The factor is of considerable importance to clinicians since it is apparently required by commonly used clinical tests. The present study found a fractionization loading of .43 for the Wechsler Block Designs and of .49 for the Arthur Stencil test. Each of these tests requires the subject to "pull-the-design-apart" (the fractionization process) in order to know which blocks or stencils must be used.

A program of the sort in which we at Penn State are engaged covers years of work. Our first contribution was the paper by Laura Murphy in 1936 based on her factor analysis of mechanical aptitude tests and verbal and non-verbal tests of intelligence. Ever since her wo k we have been critical of the common practice of classifying intelligence tests into verbal and non-verbal or performance categories, because she showed that this classification has no relationship to the factors being measured. We hope to continue the program until the challenge which Spearman gave to Binet's "avowed followers" to build a test which would measure the separate abilities at each age has been met. As a former student of Lewis Terman, who had great admiration for Binet, I feel that I can rightly be included among those to whom the challenge was given. With the aid of my own students, at least an attempt is being made.

*SECTION OF ANTHROPOLOGY**

THE ROLE OF MAN AND WOMAN ON TRUK: A PROBLEM IN PERSONALITY AND CULTURE†

By THOMAS GLADWIN‡

This paper is concerned with one aspect of a larger study, shortly to be published,[1] for which the field work was done on Truk in the Caroline Islands of Micronesia in 1947–48, and more particularly on the small island of Romonum in the Truk atoll. Romonum has a population of only about 230 but is in most respects representative of the larger population of about 10,000 which inhabits the complex of volcanic and coral islands of Truk. Although the field work proper occupied only seven months I remained on Truk until 1951 in an administrative capacity. The analysis of the material was then undertaken in collaboration with Dr. Seymour B. Sarason, Associate Professor of Clinical Psychology at Yale University.

A central feature of this study was the use of the projective Rorschach and Thematic Apperception Tests. This is not in itself unusual, for the use of these tests has, of recent years, become commonplace in the investigation of personality and culture in non-European societies. The ap-

* The Section of Oceanography and Meteorology held a meeting on May 26, 1953 at which a film, entitled "On Land and Beneath the Sea in the Hawaiian Islands," was shown by Doctor and Mrs. Jerome Schweitzer. No abstract of Doctor Schweitzer's paper has been received.

† This paper was presented at a meeting of the Section on May 25, 1953. The research reported here was supported by the Office of Naval Research, Department of the Navy, through the Pacific Science Board of the National Research Council, and was further assisted by the Wenner-Gren Foundation. It was part of the Coordinated Investigation of Micronesian Anthropology (CIMA). The group on Truk comprised a linguist and four anthropologists, headed by Doctor George P. Murdock, all of Yale University.

‡ Research Associate, Human Resources Research Office, The George Washington University.

proach in this case differed, however, in two important respects from that previously undertaken.

Firstly, although the study included a full ethnographic account of individual development as well as other aspects of the culture and fairly adequate life histories of all the persons tested, the test analyses were taken as the primary source of hypotheses both as to individual and more general Trukese personality characteristics. It has been more common to use these and other tests as a secondary source of information—a check on or supplement to other types of data—or else as a shorthand measure of personality in lieu of more exhaustive investigation.

Secondly, Dr. Sarason's interpretative approach to the projective tests differs significantly from that customarily used in studies of non-European peoples and indeed from that often used with tests of persons in our own society. He places little reliance on the conventional Rorschach scoring categories or on any standardized schema of TAT themes, the reason for this being that, even for European subjects, the validity of such criteria in predicting overt behavior from the tests is questionable and, for other societies whose members have different life experiences and recognize different behavioral norms, the ascription of any specific significance to these criteria is distinctly hazardous. Instead, Dr. Sarason views the test situation as a sample of behavior in a novel and problem-solving situation and undertakes to predict, from the subject's response to this task, his behavior under other specified life conditions. Because maturation, even in a stable society, presents to the given individual a series of novel problems which he must face as he grows up, the novelty of the test situation actually increases rather than reduces the value of the test as an indicator of adaptability in the individual.

The tests were administered to 23 subjects, 12 men and 11 women, the series being selected with an eye to the inclusion of both "average" and somewhat atypical persons, but none who were psychotic or otherwise not fully participating members of the society. Autobiographical life histories and dreams the subject could remember were also recorded in each case. In analyzing the test records, Dr. Sarason had access to none of the ethnographic or life history data. I told him only the subject's age, sex and reaction to the test situation—whether he was under obvious tension and how it manifested itself, when he laughed or made some other non-verbal response, and so on. When Dr. Sarason's analyses were completed his conclusions were examined in detail for the degree to which they were congruent with the evidence from the ethnographic and life history data, a process which was also followed as far as possible in treating of the individual cases. The high order of correspondence discovered is a considerable tribute to Dr. Sarason's method and insight. The discrepancies could all be resolved and their resolution was often quite revealing methodologically. It is obvious that, had the test results not proven rich in hypotheses or had these hypotheses not been found verifiable on the basis of our other evidence, the study would have been of some negative interest methodologically but of little substantive value.

Of course, even if the tests had proved a disappointment, or indeed had not been administered at all, the ethnographic and life history data would have remained available for analysis along the lines followed, for example, by Abram Kardiner with the Alorese material,[2] a study which indeed provided the starting point from which the design for the present one was developed. Without, however, intending any reflection on Dr. Kardiner it is my contention here that without the test results, especially the Rorschach, available for guidance, the analysis of the other data would have been subject to errors of considerable magnitude in this study of the Trukese. This contention can perhaps be best documented by a discussion of the analysis of the roles and pyschological adjustment of men and women.

An anthropolologist or any other foreigner in contact with the Trukese is immediately impressed by the degree to which the men are dominant in the society. One quickly becomes convinced that the women are subservient, insecure, and afraid to express themselves in the presence of their lords and masters. Granted there are occasional episodes which may give one pause in regard to this stereotype but they are overwhelmed by the weight of contrary, though superficial, evidence. Thus, it is a man who heads the household group, and the oldest man in the lineage (the principal functioning kin group) is the final arbiter of the affairs of its members. All political power and most esoteric knowledge is in the hands of men. In the adulterous liaisons which seem to preoccupy most Trukese during their active adult years, it is always the man who takes the initiative while the woman remains at home presumably awaiting

with eagerness a nocturnal visitor or a letter of assignation. Breadfruit is the most important staple food of the Trukese, both actually and psychologically, and its collection and preparation is almost exclusively a male task, since women can neither climb the tall breadfruit trees nor undertake the arduous job of pounding the steamed breadfruit to proper consistency. Similarly, women are felt to bring bad luck on any fishing expedition which goes beyond the immediate offshore reefs of the island. Men beat their wives with impunity and in general extend to them little consideration and few courtesies. It is perhaps justifiable in the light of this array of evidence that, after four years of constant association with the Trukese, I still brought back with me the belief that the man is in a securely dominant position in the society.

Then Dr. Sarason analyzed the Rorschachs, and after them the TAT's, and in both cases concluded that the men were notably more anxious than the women and would tend to respond less adequately in any situation of conflict or doubt. Even in the areas of sexual activity and food production and distribution, where men play a dominant role and appear to hold all the initiative, he found the women using these activities as a means of subtle aggression toward men and as media for expression of their own assertiveness—a conclusion which he drew from the male protocols even more clearly than from those of women.

I must confess to having viewed Dr. Sarason's results at this time with somewhat more skepticism than purely scientific caution demanded, but of course reviewed the data available to see what support could be adduced for his conclusions. One striking piece of evidence lay in suicide, an act which is in many ways the ultimate expression of a sense of inadequacy in the face of conflict. Although I recorded only a few cases of genuine attempts (none of them successful), in every one of these the protagonist was a man. I then turned to the several accounts I had of conflicts which arose over the selection of marriage partners. This is an area in which the Trukese culture is notably inconsistent, favoring on the one hand the principle of marriage arranged by the parents and lineage elders, and on the other the right of young people to select their own spouses. With the cultural norms themselves in conflict it is evident that a difference of opinion in this regard can only be resolved at the personal level, and here I found that in every case where effective resistance was made against the wishes of the elders, it was the girl rather than her prospective husband who opposed them.

While suicides and major conflicts between generations over marriages are exceptional on Truk, the data on these subjects provided such clear-cut evidence of differential male–female reactions in situations of severe conflict that their support for Dr. Sarason's hypothesis was more than inferential. It appeared justifiable, therefore, to re-examine the data on more usual behavior to determine whether it was possible to conclude that Trukese men are actually more anxious than women and, more particularly, to attempt to discover the basis for such anxiety among men. We may take as examples of this analysis the status of men in the household and their role in adulterous liaisons, both of which we cited above as apparent instances of masculine dominance. As will become evident when the data are viewed from this standpoint, it is clear that Dr. Sarason's hypothesis of masculine insecurity is not only plausible but throws into clear perspective the greater problems of adjustment faced by the Trukese man compared to the woman as they grow into maturity in their society.

There is little evidence that there is any substantially differential treatment of boys and girls by adults until later childhood and, even at this time, the differences lie primarily in the demand for the wearing of appropriate clothing and learning the tasks proper to their sex. At puberty, however, the fact that brother and sister (real or classificatory) are sexually mature by cultural definition requires that they no longer live in the same house, and it is the boy who has to leave his home. In the past there were men's houses to which he could go and which thus softened somewhat the blow of being forced to leave his home. At present the men's houses are no longer maintained and a boy has to live as something of an outsider in some relative's household or, in some cases, sleep in another room or on the porch of his own house, if it is large enough, but even if he thus remains at home it is only on sufferance. Meanwhile, a girl entering correspondingly upon her womanhood remains a member in good standing of the household in which she has been brought up.

After a few years of adolescence, during which a youth has no home he can really call his own, but during which he has increasingly to share the

food-producing labors of whatever household he lives in, he marries a girl of his own or his parents' choice as the case may be. But, in either case, he usually goes to live in the household of his wife, for the society of Truk is predominantly matrilocal. Here he has a more assured and rightful place in a household, but the people with whom he lives and with whom he works most of the time are his wife's relatives, not his own. Furthermore, his acceptance and retention in the household is contingent on his good behavior toward her relatives and in large measure on his continuing contribution to their food supply. If he finds his situation distasteful he may leave, but he still may not return permanently to his own home where there almost always lives a sister of some sort. On the other hand, the woman he has married is of course still living in her own home and has now improved her status substantially by bringing in and retaining within the household another working member whose contribution to the family larder and family enterprises is mediated through her. Even if she does very little work of her own, a condition which obtains for about two years every time she has a child, she is still producing her share of food through the labors of her husband.

Thus, although the fact that only a man is able to be self-sufficient in producing all the necessary kinds of food (as long as he retains his strength) would appear at first glance to place him in a more secure position than a woman, this actually has the opposite effect. Because he *can* do this work he finds that he *has* to do it in order to maintain his position in the household, while the woman who is not felt to have his abilities is also not burdened with his obligations. A man consequently becomes anxious if any threat arises to his physical capacity, a fact which is graphically illustrated in times of illness. If a woman becomes sick, she tends to react appropriately to the gravity of her illness. A man on the other hand with only a mild stomach ache or a slight fever and headache succumbs immediately to querulous despair. He takes to his bed and covers himself with a sheet to the accompaniment of moans, groans and demands for sympathy and attention. Significantly, however, men who are injured even quite seriously (provided there is no danger of their being permanently crippled) continue to go about and perform such tasks as their injury permits. In the case of injuries, recovery is only a matter of time and poses no threat of long-standing incapacity.

Turning now to the adulterous liaisons which so many Trukese consider of absorbing importance, we find the same contrast between apparent masculine dominance and the actually more secure position of the woman. Sexuality and the attitudes of the Trukese toward it form a complex and ramified problem we cannot fully explore here but, in essence, in adultery the initiative lies almost wholly with the man but so do the risks, both physical and psychological. Most liaisons begin either with a secret letter or the man slipping silently into the woman's house and awakening her. In both approaches he runs the risk of discovery and serious trouble, while the woman if she is at all adroit can protest her outraged innocence and join in the hue and cry against her would-be (and perhaps actual) lover. Even if he is not discovered, the man may be rejected by the woman, a considerable blow to his self-esteem but a matter of little consequence to the woman for he will probably not advertise his failure and hence her lack of receptiveness. Finally, if he does succeed in his approach and persuasion and the couple have intercourse, he can only feel himself successful if he brings his partner to her climax. Many Trukese phrase intercourse explicitly as a contest, but it must be noted that in this contest it is only the man who can lose and not the woman. Indirectly the woman is also vulnerable, for some women are felt to be "quicker" and hence more desirable than others, a matter of considerable discussion among the more profligate males, but this long-term effect can do little to assuage a man's injured pride in the face of his failure.

In sum, then, on the basis of this and other evidence it became increasingly clear that Dr. Sarason's conclusions, based exclusively on his analysis of the projective tests, provided a substantially more economical and inclusive explanation for the data I had collected on the roles of men and women, in spite of the fact that, after four years of contact with the Trukese, I continued to assume that the superficial dominance of men in the society reflected also a corresponding sense of greater security at the psychological level. Of course, it can be remarked that in this paper I have more than anything else documented my own naiveté. In large measure this is true, but it is precisely here that our purpose in designing the study in the way we did becomes clear. No anthropologist or psychologist is omniscient and, where I failed to perceive what seems in retrospect an obvious relationship, others could have

been equally blind to this or other aspects of the data. The use of independently and adequately interpreted projective tests in the study of personality and culture can serve others, as it served me, in forcing a re-examination of data with which it is easy to become all too familiar, and in exposing previously unsuspected relationships within the data which can then be further explored. On the other hand, by no means all of Dr. Sarason's conclusions stood up unmodified under examination and he would be the first to admit that some were simply wrong or at least inadequate. While we can strongly urge, therefore, the more extensive use of projective tests in conjunction with the collection of as many other sorts of information as possible in the study personality in other societies, equally we should warn against the unreserved acceptance of test interpretations alone as a means of arriving at a description of the psychological attributes of the people under study.

Literature

1. GLADWIN, T. & S. B. SARASON. Truk: Man in Paradise. Viking Fund Publications in Anthropology. In press.
2. DU BOIS, C. 1944. The People of Alor. Univ. of Minnesota Press. Minneapolis, Minn.

CONFERENCE HELD

SECTION OF BIOLOGY

"THE COLON: ITS NORMAL AND ABNORMAL PHYSIOLOGY AND THERAPEUTICS"

MAY 8 AND 9, 1953

The Section of Biology held a Conference on "The Colon: Its Normal and Abnormal Physiology and Therapeutics." Doctor Thomas P. Almy of the Cornell Medical School, Cornell University, New York, N. Y., was the Conference Chairman in charge of the meeting.

The program consisted of the following papers:

Friday, May 8

PHYSIOLOGY AND PHARMACOLOGY OF THE COLON

Morning Session. Chairman: Thomas B. Almy.

"Normal Physiology of the Colon in Animals," J. Paul Quigley, University of Tennessee, Memphis, Tenn.

"Autonomic and Pharmacologic Control of Colonic Activity," George B. Koelle, University of Pennsylvania, Philadelphia, Pa.

"Normal and Some Abnormal Colonic Motor Patterns in Man," Charles F. Code, Mayo Clinic, Rochester, Minn.

"Action of Autonomic Drugs on the Human Colon," Fred Kern, Jr. University of Colorado, School of Medicine, Denver, Colo.

"Roentgen Appearance of the Normal and Subnormal Colon," Sydney Weintraub, Cornell Medical School and New York Hospital, New York, N. Y.

ETIOLOGY OF COLONIC DISORDERS

Afternoon Session. Chairman: Franklin Hollander.

"Childhood Conditioning and Colonic Disorders," Dane G. Prugh, Harvard Medical School, Boston, Mass.

"Functional Colitis and Constipation," Sara M. Jordan, Lahey Clinic, Boston, Mass.

"Congenital and Acquired Serious Obstipation," Keith Grimson, Duke University Medical School, Durham, N. C.

"Physiological and Psychological Factors in the Production of Colitis," William J. Grace, Cornell University Medical College, New York, N. Y.

"Physiological and Psychological Factors in the Production of Constipation," Thomas P. Almy, Cornell University Medical College, New York, N. Y.

Saturday, May 9

PHARMACOLOGY OF THE ABNORMAL COLON

Morning Session. Chairman: McKeen Cattell.

"Pharmacology of the Stimulant Laxatives," Janet Travell, Cornell University Medical College, New York, N. Y.

"The Statistical Design of Aperient Studies," John C. Seed, Sterling-Winthrop Research Institute, Rensselaer, N. Y. and Raymond Harris, Ann Lee Home, Albany, N. Y.

"Quantitative Comparison of Bulk Laxatives," Maurice L. Tainter, Sterling-Winthrop Research Institute, Rensselaer, N. Y.

"Clinical Comparison of Bulk and Stimulant Laxatives," Leo J. Cass, Harvard University, Brookline, Mass.

"Antispasmodic Therapy for the Hyperactive Colon," Alfred P. Ingegno, Brooklyn, N. Y.

"Antilysozyme and Steroid Therapy in Colitis," Seymour J. Gray, Harvard Medical School, Boston, Mass.

THERAPEUTIC MANAGEMENT OF COLONIC DISORDERS

Afternoon Session. Chairman: Sara M. Jordan.

"Natural History of Ulcerative Colitis," Benjamin White, Yale University School of Medicine, New Haven, Conn.

"Clinical Management of Colitis," Thomas C. Machella, Hospital of University of Pennsylvania, Philadelphia, Pa.

"Clinical Management of Constipation," Franz J. Ingelfinger, Boston University Medical School and Evans Memorial Hospital, Boston, Mass.

"Changes in Colonic Function and Use of Laxatives in the Aged," Henry Rafsky, New York University, and Lester J. Honig, Lenox Hill Hospital, New York, N. Y.

"The Problem of Constipation in the Institutionalized Patient," Robert C. Batterman, New York Medical College, New York, N. Y.

"Proctological Aspects of Constipation and Diarrhea," Robert Turell, New York, N. Y.

The Section of Biology provides conferences for active workers in the special fields of Biology. Attendance is limited to those invited to participate in these conferences and to interested Members of the Academy.

NEW MEMBERS

Elected May 15, 1953

LIFE MEMBERSHIP

Wilson, William S., Ph.D., Physics. Associate Director, Geophysical Institute, Head, Department of Chemistry, University of Alaska, College, Alaska.

SUSTAINING MEMBERSHIP

Mulford, Dwight James, Ph.D., Biochemistry. Associate Professor, Kansas University, Lawrence, Kansas.

O'Leary, Paul Arthur, M.D., Dermatology, Syphilology. Head of Section on Dermatology and Syphilology, Mayo Clinic, Rochester, Minn.

Robinson, William Witten, M.D., Endocrinology. Director of Clinical Research, Rockwood Clinic, Spokane, Wash.

ACTIVE MEMBERSHIP

Adams, William Robert, M.D., Pathology. Intern, Department of Pathology, Yale University, New Haven, Conn.

Anderson, Frank David, Ph.D., Anatomy. Instructor in Anatomy, Cornell University, Medical College, New York, N. Y.

Anderson, John Walberg, M.S., Histology, Embryology. Graduate Assistant, Department of Zoology, Cornell University, Ithaca, N. Y.

Astrup, Tage, D.Sc., Biochemistry, Physiology. Carlsberg Foundation, Biological Institute, Copenhagen, Denmark.

Baer, Walter, M.D., Medicine. Private Practice, St. Paul, Minn.

Baker, Frances, M.D., Physical Medicine. Private Practice, San Mateo, Calif.

Barton, Ambrose Donald, Ph.D., Biochemistry. Post Doctoral Fellow, McArdle Memorial Laboratory, University of Wisconsin, Madison, Wis.

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